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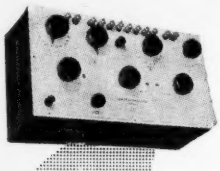

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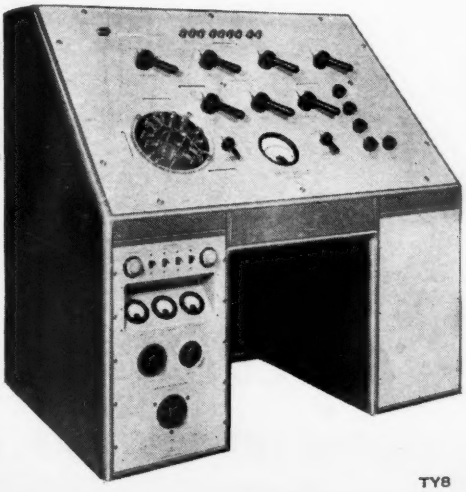

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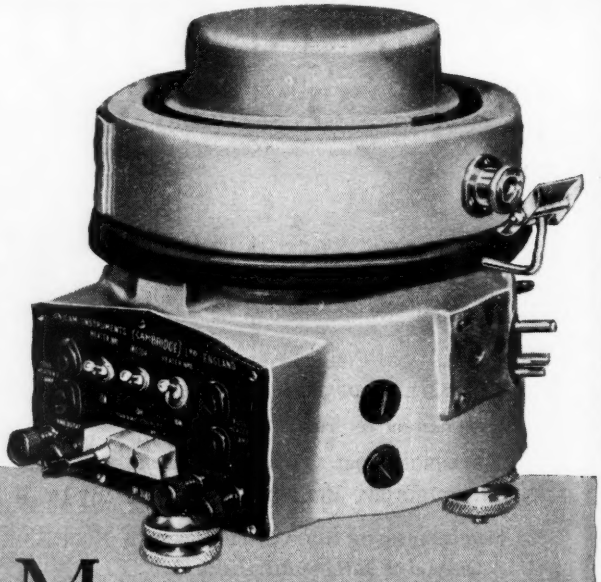

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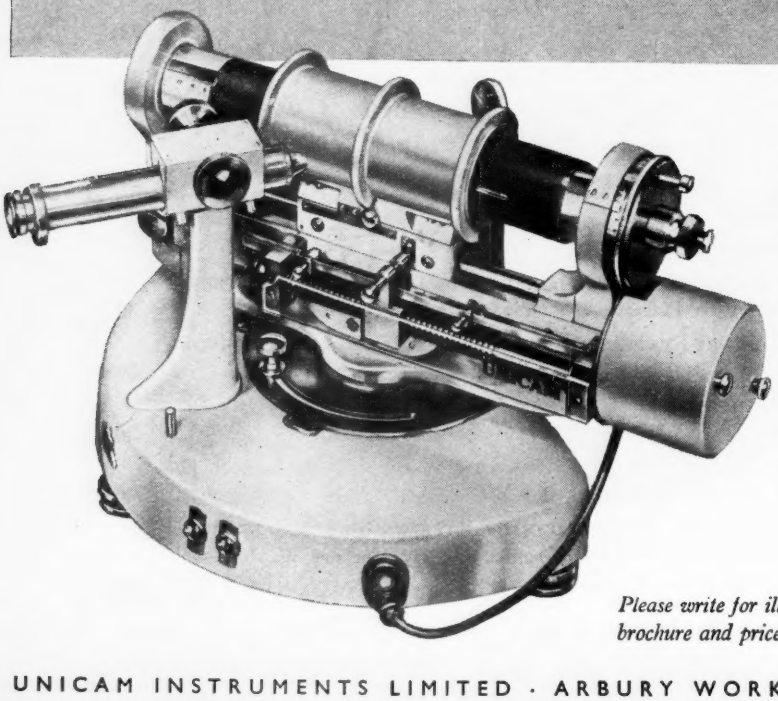

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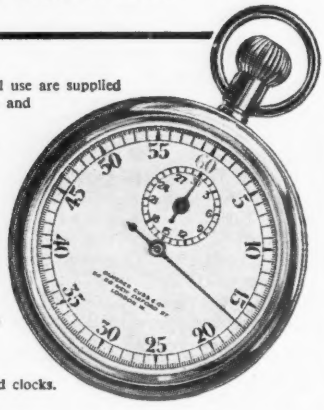

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POSITION AND FUNCTIONS OF THE RESEARCH ASSOCIATIONS

IN its first annual report last April, the Council for Scientific and Industrial Research said that it was undertaking a comprehensive study of the way in which the Department assists the promotion and application of science in industry in Britain through the work of grant-aided research associations. Apart from indicating that the Council was prepared to consider to what extent industries served by the research associations could be expected to shoulder the whole financial burden of an adequate research programme either now or in the near future, the report made no reference to immediate action. The Industrial Grants Committee of the Council, however, has been surveying both the value of the work of the associations and the methods used to assess and administer the Government grant; and Government grant policy in relation to research associations is to be reviewed during the present year in the light of this survey.

Mr. John Wilson, lately director of research, British Rayon Research Association, afterwards took grant-aided research associations as the subject of his Hinchley Memorial Lecture to the British Association of Chemists on October 29, but was much more constructive than Dr. N. de Bruyne, in an article in *The New Scientist* (3, 28 (78); 1958), to whose criticism he referred, as well as—though not quite accurately—to that of Dr. H. Levinstein. Mr. Wilson's lecture was indeed concerned primarily to clarify ideas as to the real functions of the grant-aided research association, and though he too recognized that the research association is at a disadvantage as compared with the research department of the individual firm in selecting targets in technological research, he fully recognized that there is a definite place for co-operative research. Quite apart from its function in providing for co-operation in the attack on common problems and perhaps particularly on problems demanding very costly equipment or resources beyond those of individual firms, Mr. Wilson maintained that there is a definite place for the research association in fundamental research, particularly the systematic disciplined exploration of a specific field, which is irreconcilable with the complete freedom of action of the universities.

The whole subject of co-operative industrial research was carefully surveyed by Prof. R. S. Edwards in his book, "Co-operative Industrial Research", published in 1950. Prof. Edwards did not indeed examine the essential functions of the research associations, the broad fields of research they should pursue or their relations with the universities. His analysis was concerned with their economic aspects and, as Dr. de Bruyne noted, he advanced convincing arguments against the compulsory levy; though Dr. de Bruyne ignored the broad conclusion reached by Prof. Edwards both on his statistical evidence and on his inquiries into industrial opinion, that the research association is a sound industrial institution.

This is obviously the view of Mr. Wilson. He argues, indeed, that if research associations are used to attack problems common to groups of firms, the formation of a new research association will, in fact, lead to economy in the use of scientific man-power. Whether, however, it is cheaper to attack common problems on a co-operative basis or for individual firms to act independently and simultaneously depends on more than the selection of objectives which are worth while. It pre-supposes appropriate conditions of service, staff of similar high quality, and management capable of terminating an investigation at the right moment, as well as contacts between the research department and production which will ensure that the results are utilized as effectively when they come from the research association as they are within an individual firm.

There are questions of incentive and judgment involved here, and also of location, which can have a vital bearing on efficient communication, and these factors deserve more examination than they have yet received. Mr. Wilson and Dr. de Bruyne gave them no more attention than did Prof. Edwards; but it is essential that they should be duly weighed in the re-assessment of the work of the research associations on which the Council of Scientific and Industrial Research is now engaged. Mr. Wilson makes an important point here in regard to staff, when he remarks, in urging that the research associations should give more attention to fundamental research, that the method of approach and type of man required for such work are quite different from those required in applied research. The difficulty can be over-stressed, for it is recognized in industry and has been overcome.

Much more important is the clear recognition of the relation between the research associations and the universities and also the research stations of the Department of Scientific and Industrial Research. This is probably essential in any event in order to check the danger of a research association becoming absorbed excessively in technical service, as distinct from research. A clear picture of the general structure of Britain's research effort and the respective functions of the universities, the independent research institutions, industry itself, the research associations and the Government research stations is necessary for the wise apportionment of effort and resources, and would contribute also to flexibility. It bears closely on the application of results, which is not simply a matter of the technique of communication but also, and sometimes mainly, of psychology.

Here, in touching on the communication of results, Mr. Wilson insists that the research associations must

give some attention to information work; he suggests, nevertheless, that this function should be centralized in the Department's own Intelligence or Information Division. That is a debatable issue, too large to be discussed here; for it is, indeed, a facet of the library and information services of Britain as a whole, though it might be observed that Mr. Wilson's suggestion that such a step would release funds for research illustrates the way in which Government failure since the War to develop those services adequately has led to waste of effort and resources in research. Mr. Wilson is right, indeed, to point out how easy it is—and not only in the research associations—for resources to be diverted from the primary purpose and frittered away on secondary issues, even though these may be important. He mentions also education, and, valuable as has been the contribution of the research associations to technological education, this is not in itself a reason for their existence, and their research functions should be evaluated independently. Once the function of any particular research association has been clearly established and approved and the extent of the necessary resources determined, its ancillary contribution to technical education can be readily assessed. This should never be allowed to interfere with research itself, and it would be better to charge any direct loss involved to technical education as such, rather than allow it to appear in the research budget, unless it is impossible or impracticable to separate the two.

The main issue, however, is the proper function of the research association in Britain. Should it be, as Sir Lawrence Bragg suggested in 1942, an institution with a nucleus of permanent staff and with specialized and expensive equipment, but in the main with a constantly changing staff—a place to which men go for periods of intensive work, both from industry and the universities? Clearly, Mr. Wilson aimed at meeting the objection which Sir George Thomson advanced against Government research in his Fawley Foundation Lecture, in order to ensure the mobility which prevents fossilization. Mr. Wilson recognizes the value of the suggestion, but points out that success would depend on the existence of a virile organization in full operation, with a character, tradition and individuality of its own.

Mr. Wilson claims that the research associations possess the requisite resources for successful fundamental research, which requires the simultaneous impact of different disciplines, and can provide an atmosphere of freedom of discussion and publication which it is virtually impossible for the research department of an industrial firm to provide. He points out that, unlike the universities, they can accept directives from industry; but he suggests that their present low output of fundamental research could well be stimulated by administrative action in fixing the terms of future grants. He does not refer to the possibility that they might offer some contribution to the problem which the increasing cost of specialized equipment for research raises, and he does not mention the problem of continuity of finance.

These, however, are important factors on which, among others, the decision as to the form in which research for any particular industry should be prosecuted should depend. Conditions may vary widely from one industry to another, and although there seems to be a sound *prima facie* case for continuing in Britain the system of research associations in general, that is not to say that the existence of every research association is automatically justified. Each needs to be examined in relation to its own particular circumstances, and in relation to the other institutions which may serve that particular industry's need.

Finally, while the survey of the research associations and their work and functions on which the Council of Industrial and Scientific Research is now engaged is particularly welcome in view of the recent announcement that the Department's resources are to be substantially increased over the next five years, it is important that this re-assessment should be made in relation to the functions of the Department's own research stations and of the universities themselves. Sound decisions cannot be made in isolation; and while the expansion of the Department's resources makes it more desirable than ever that the Department's organization and functions should be periodically re-assessed as independently as possible in relation to present needs and conditions, it is essential to realize that the Department is only a part of the nation's research effort. The repercussions on other sectors of the nation's effort need to be considered also; nor should the large issue of the administration and control of research be ignored.

PAPERS AND CORRESPONDENCE OF C. S. PEIRCE

Collected Papers of Charles Sanders Peirce

Edited by Arthur W. Burks. Vol. 7: Science and Philosophy. Pp. xiv+415. Vol. 8: Reviews, Correspondence and Bibliography. Pp. xii+352. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1958.) Each 63*s*. net.

ON the east of the Atlantic, all too little is known of the breadth and profundity of this American philosopher's work. In the course of his life (1839-1914), Peirce touched upon many problems well in advance of his time: to read his papers to-day is to see what a considerable prophet he was. Events have not hitherto been too kind to his memory: six volumes of his writings appeared between 1931 and 1935, after which, for a variety of reasons, nothing more was done until 1954, when Harvard University initiated new progress, and these two volumes are the result. They are most welcome.

The earlier work of Peirce consisted of studies in deductive, inductive, and symbolic logic; now these are joined by contributions to science and philosophy, with some reviews and correspondence. The salient feature of this immense activity is the combination of an enviable analytic power with sturdy practical experience. The latter came about because Peirce spent some thirty years in the service of the United

States Coast Survey, investigating astronomical and geodetic questions. In that sense he was a precursor of the distinguished school established in Britain by the late Sir Gerald Lenox-Conyngham at Cambridge. To read these papers, written in the early eighties, on the reasons for, and limitation of, pendulum experiments is to gain a substantial insight into the physics of the Earth. Peirce must have possessed something of a flair for discerning the essentials of his subject.

However, it is as a philosopher that Peirce will be remembered: his views on what to do and how to do it (or how not to do it) tend to be devastating when applied to research in general. He provides discriminants for the worth-whileness of tackling any given set of propositions experimentally. These criteria concern priorities, time, cost, and so on, and seem close to the considerations which any responsible director of research would apply to-day. In his time, naturally, the play was not for the high stakes of the mid-twentieth century, but the theory is all there, and of great interest. Another example of his rapier-like mind is his approach to the old game of 'twenty questions'. On his showing, a score of interrogations is far too generous an allowance; something like half that number should reveal almost anything, if every shot carries the maximum possible epistemological weight. All this points inevitably towards the growth of a new science, that of cybernetics and information theory, utterly unknown during Peirce's life-time, but destined to become of major importance hereafter. If alive now, he would be a master of electronic computers. Another example of his power of foresight, as Prof. A. D. Ritchie has noticed, is his grasp of the influence of order of magnitude upon the laws applicable to single particles on one hand, and to assemblies on the other. Taken for granted to-day, Peirce perceived this more than sixty years ago.

The collection of reviews and correspondence (strengthened by a detailed bibliography) portrays Peirce as a distinguished reviewer, kindly but ruthless in pressing an author's meaning to the limits of both philological and semantic significance. He seems to extract from a book considerably more than the writer probably intended to put into it. This applies more particularly to minor works, but when dealing with a major theme like Lobachevski's "Geometrische Untersuchungen", his comments are masterly.

It may be useful to indicate the plan and contents of the bibliography, more particularly since much of the material had its origin in rather unlikely places. The general scheme is: (1) Science of Discovery, (2) Science of Review, (3) Practical Science and Miscellaneous, (4) Book Reviews, (5) Life and Letters. This is, in fact, the author's own pattern of taxonomy, and depicts once again the unique quality of his mind. It should perhaps be added that a number of unpublished manuscripts still remain, which are of value for an assessment of his place in the development of philosophy in America. The general bibliography covers the period 1860–1911, with the addition of a few undated entries. Another section follows, containing items from *The Nation*, including Peirce's review of the two volumes of J. Willard Gibbs (1907). Lastly, comes a list of miscellaneous entries and indexes (cross-references, proper names and subjects). If ever a work deserved the time-honoured title of the "Theory of Knowledge" these volumes certainly do. The editor and the Harvard University Press are to be congratulated upon their efforts.

F. I. G. Rawlins

THE KILLING OF MICRO-ORGANISMS

Disinfection and Sterilization.
By G. Sykes. (Spon's General and Industrial Chemistry Series.) Pp. xviii+396+7 plates. (London: E. and F. N. Spon, Ltd., 1958.) 75*s*. net.

THIS book is a survey of the physical and chemical methods of killing micro-organisms. The scope is wide and the author, who has purposely avoided the subjects of chemotherapy and antibiotics, has condensed the material into 370 pages. Although this has prevented any detailed discussion it has permitted some cases of repetition in different sections of the book.

The subject-matter can be divided into two main sections, the physical and the chemical methods (sterilization and disinfection). Parts 1 and 5 of the book deal with the chemical methods and Part 2 with the physical ones. The disinfection and sterilization of air (Part 3), the disinfection of viruses (Part 4) and preservatives (Part 6) are treated separately.

Part 1 deals with the theory of disinfection and methods of testing. In the brief section on bacterial enzymes the difficulty of differentiating between disinfectant action and chemotherapeutic action has forced reference to some chemotherapeutic agents.

The treatment of the dynamics of disinfection is brief and no mention is made of the probit percentage survivor/time curves used by many modern workers, including Jordan and Jacobs, who are so often quoted in this section.

Details of many of the methods of testing are stated although little is given on the comparison between the methods, or with other methods of biological assay.

The chapters in Part 2 deal with sterilization by heat, radiations, filtration, gases and vapours, and the effects of cold, desiccation, and cellular disintegration. Each chapter is complete in itself, but the reader will have difficulty in finding which is the most efficient method for sterilizing a specific material.

Part 3 is a concise and well-balanced account of the disinfection and sterilization of air.

Most of the short section on disinfection of viruses could be incorporated in other sections of the book since all the virus-inactivating agents mentioned in this section have more detailed treatment in other sections.

In Part 5 the commonly used disinfectants are discussed under the headings: phenols and related compounds, dyes, quaternary ammonium compounds, halogens, and heavy metals. The sections are comprehensive but, although the coal tar disinfectants constitute the most widely used group, the problems associated with their formulation receive but little consideration. The testing of 'quats' would be more appropriately discussed in Chapter 3.

It is essential that any book on disinfection should discuss the problems of microbial spoilage, and Part 6 deals adequately with this, and quite correctly other causes of spoilage are not mentioned.

The book is well indexed and the bibliography is extensive, although the practice of referring to a 'private communication', which has not been made or is not going to be made freely available, is very frustrating.

It will be a valuable reference volume in all microbiological libraries.

A. M. Cook

REFLEXIONS ON THE MOTILITY OF MUSCLE

The Motility of Muscle and Cells

By Prof. Hans H. Weber. Pp. vii + 69. (Cambridge, Mass. : Harvard University Press ; London : Oxford University Press, 1958.) 28*s*. net.

PROF. WEBER'S book might aptly have borne the sub-title '*multum in parvo*', for in 58 well-spaced pages of text he not only describes the biochemical basis of motility in striated and smooth muscle and in a number of unicellular organisms, but also puts forward a theory of the transformation of chemical energy into work during the contraction process. So far as the descriptive passages are concerned, there can be nothing but praise, particularly for the elegant techniques used in investigating the specialized movements of cells in mitosis, of fibroblasts and of *Vorticella*. Doubts quickly arise, however, when we consider the theoretical interpretation of the highly specialized contraction of striated muscle.

Prof. Weber sets out "towards the unknown region" from the postulate, well supported by innumerable experiments, that the energy for contraction is derived from the splitting of adenosine triphosphate by the actomyosin of the myofibrils in the presence, specifically, of magnesium ions, whereas no energy is required for relaxation, which is a passive process brought about by adenosine triphosphate, when its splitting is inhibited. To this he adds the further postulate that the physical changes of state take place according to the muscle model of Hanson and Huxley. This may be visualized, in the sense used by Prof. Weber, by considering the sarcomere at rest as a collapsible compartment, bounded by the *Z*-disks to each of which is attached a set of rigid pistons, the actin filaments, sliding freely within a centrally disposed cylinder, the *A*-band, made of parallel rigid filaments of myosin. During contraction, the pistons are envisaged as being pulled forcibly into the cylinder, rather in the manner of an iron bar into a solenoid. The problem is then to explain the nature of the attractive force in terms of these two premises. This Prof. Weber does by assuming the reaction occurs in steps, each of which consists of (*a*) a phosphorylation of a group on the actin piston, (*b*) the interaction of this group with a group on the myosin 'cylinder', resulting in dephosphorylation, and (*c*) further interactions between the dephosphorylated group and groups on the cylinder, each of which has a higher equilibrium constant than the one preceding it, or as Prof. Weber puts it, a lower standard energy in the temporary bonds formed. Attractive as this idea may be, it seems to be founded rather insecurely, because the results of Ulbrecht and Ulbrecht on which the very first interaction is based show that the postulated phosphorylation of protein by adenosine triphosphate occurs only with natural, and not with artificial, actomyosin, in spite of the fact that either sort of protein will form equally contractile threads. Apart from this objection, there is also the question of how sufficient of the energy derived from the primary phosphorylation is stored in the actin group to be available for the later interactions, without also postulating a complex series of configurational changes in this actin group, in other words, entropy changes, all of which must be reversed before the next step can take place.

When we pass from the steps to the integrated movement of the whole sarcomere we encounter another difficulty which has apparently escaped the notice of the adherents of the piston-and-cylinder model. That is, the question of how heat is given out during shortening. As is well known, Prof. A. V. Hill has been at great pains for many years to demonstrate once and for all that the total energy of contraction *in tres partes divisa est*, of which the first is the heat of activation, liberated explosively almost as soon as the stimulus arrives, and found to be constant and independent of length or load ; the second is the heat of shortening, which is a linear function of the degree of shortening and is also independent of load, and the third is the work done. The latter two make up the so-called extra energy which can appear only if the muscle is allowed to shorten and do work, and which is entirely absent from the ideal isometric twitch. Now, Prof. Weber's scheme does not take into account the first part of this energy release, the heat of activation, presumably because it is difficult to explain by simple thermochemical reasoning from this model or any other. It has been likened by some workers to a charging-up process in contrast to shortening, which is a discharge process ; but if this were so, and no entropy changes were invoked, it would mean that more or less charged molecules would be left over at the end of a twitch, depending on whether the shortening were restricted or free. What would happen to the heat then ?

Secondly, reasoning from the model, Prof. Weber concludes that it is unique in satisfactorily explaining the origin of the heat of shortening ; but this is only so if further restrictions are applied. For example, the scheme shows only one active group of actin interacting at each step with four or more groups of myosin, although it is implicit that there are others behind it on the free part of the piston. If so, two courses are open : (1) interaction will occur with increasing frequency as the piston is pulled further into the cylinder, that is, as more actin groups are opposed to more of the myosin steps, or (2) the actin groups are discharged as they enter the cylinder and afterwards remain inactive. If the first, then the extra energy released in free shortening is a parabolic function of the distance moved, that is :

$$-\text{Heat} = -K\,(n^2 + n)/2$$

where K is a constant and n is the number of steps. This is clearly incompatible with Hill's equation and must be rejected. On the other hand, if the second, then only a restricted area at the end of the myosin cylinders can contain active groups. Now this is compatible with the linear heat relation, but it runs counter to generally accepted views of the orientation of the *A*-band material and, for that matter, of myosin itself. If we must reject this idea, then we are left only with its opposite, that active groups are disposed throughout the length of the myosin cylinder whereas those on the actin pistons are restricted to an area at their tips. This is a rather more plausible concept, if no less revolutionary than the other, yet it still faces us with the problem of how a long chain of homogeneous protein, perhaps extending for 10^4 angstroms, can be protected from interactions, except at its tip. Is it possible that this role is fulfilled by tropomyosin, a protein the function of which is as yet unknown ? Or must we abandon the piston and cylinder model in spite of its attractiveness in other ways ?

It is in raising these questions in such a precise and concise manner that the great merit of Prof. Weber's book lies, and while we may not agree with him over details, it is undeniable that he has successfully delimited the area where the answers will one day be found.

J. R. BENDALL

METEOROLOGICAL AND IONOSPHERIC STUDIES IN THE ARCTIC

Polar Atmosphere Symposium, Oslo, 2–8 July, 1956

Part 1: Meteorology Section. Edited by R. C. Sutcliffe. Pp. xi+341. 70*s*. net. Part 2: Ionospheric Section. Edited by K. Weekes. Pp. xiii+212. 60*s*. net. (London and New York: Pergamon Press, 1957 and 1958. AGARDograph No. 29. Published for and on behalf of the Advisory Group for Aeronautical Research and Development, North Atlantic Treaty Organization.)

DURING and since the Second World War, networks of meteorological observing stations making upper-air observations by radio-sonde have been established in the American Arctic by the United States and Canada, in Greenland by Denmark and the United States and in the Russian sector by the U.S.S.R. In addition, temporary aerological stations have operated on ice floes and observations by regular Arctic weather reconnaissance flights have been made by using 'drop-sondes' along their tracks. It appears, however, that at the time of the conference much of the Arctic aerological data not only remained unpublished but also some were unavailable. The majority of the twenty-four papers on meteorology are concerned with the analysis of the available upper-air data though, besides these synoptic and aerological studies, there are also accounts of the recent climatic changes in the Arctic, of radiation measurements, of visual range and 'whiteout' and of the distribution of ice in Arctic seas. The discussions that followed the papers are admirably reported and make interesting reading, often ranging into wider issues than those raised by the papers that initiated them. They include, for example, an exchange of views between Scherhag, Sutcliffe, Namias and Fjörtoft on the warming effect on the stratosphere which Scherhag some years ago attributed to the occurrence of a solar flare. The revelation that many local winds ascribed to steep slopes are often, in fact, unconnected with the slopes, led to a suggestion that certain classical text-book concepts require revision. A further assertion that the concepts of synoptic analysis should be included in this revision called forth an immediate and vigorous defence of frontal analysis by distinguished meteorologists.

About half the twenty-one papers presented to the Ionospheric Section deal with the methods, results and theory of ionospheric drift measurements. The discussion naturally centred on the interpretation of the results in different conditions as representing true winds or the effect of electric fields, and on the difficulty of determining the height to which the drift measurements refer. There follow papers on prediction techniques applicable to problems of polar communication, on absorption measurements and one on a theory of meteor echoes of long duration based on atmospheric turbulence. This volume concludes with a short report of a discussion of measurements of absorption associated with the solar event of February 23, 1956.

The planning of work in the Arctic during the International Geophysical Year must have been materially assisted by this meeting of meteorologists and ionospheric physicists at Oslo during the summer of 1956, though it is hinted in at least one of the papers that the concentration of activity on the Antarctic would inevitably limit the scope of the effort in the Arctic.

JAMES PATON

INTRODUCTORY ELECTRONIC THEORY

An Introduction to Electronic Theories or Organic Chemistry.

By G. I. Brown. Pp. vii+209. (London and New York: Longmans, Green and Co., Ltd., 1958.) 15*s*. net.

A PROBLEM confronting all writers of elementary text-books on the mechanism of organic reactions is the marriage of theoretical conclusions with suitable experimental evidence which is within the knowledge of the reader. Elementary text-books often have the tendency to particularize general reactions of classes of compounds in terms of the simplest members. These are seldom characteristic members of the class. The problem is a difficult one and the author has courageously, and probably rightly, avoided it by omitting the experimental material so far as possible.

This book is clearly and easily written and the argument simply stated. The usual topics are discussed, including electrophilic and nucleophilic substitution, the molecularity of reaction, hydrolysis, addition and elimination reactions, hydrogen-bonding and homolytic and heterolytic fission. The book has also a good historical introduction and very useful chapters on the nature of bonding. There is also a timely appearance of the high value of the latent heat of sublimation of carbon, evidence for which is now compelling.

As each topic is dealt with separately, the book will prove a ready source of information as well as being useful for reference. The text is clearly printed and attractively presented, the whole work being well indexed. The diagram, except for the point mentioned below, is well constructed and produced.

There are some matters with which I disagree. The discussion of the nitration of *p-ter*butyltoluene comes to an erroneous conclusion; recent evidence strongly favours a steric explanation of the experimental observations. The suggested mechanism for the Hofmann degradation (p. 193), moreover, would not meet with universal approval.

Finally, there are some trivial errors. The display of the electrons in the diagram (p. 34) suggests an interpenetration of the valency orbits of the oxygen atoms, and the failure to assign the $-NO_2$ and $-COOH$ groups to particular carbon atoms on p. 196 is confusing at first sight. Surprisingly, collinear has been mis-spelt.

In spite of these criticisms, however, I consider the book to be well constructed and deserving of serious study. If it reaches the classes of readers, sixth-form students, first-year university students and older chemists who are interested, as the author wishes, it will do much to ensure a greater appreciation of this important subject.

R. I. REED

Feedback Theory and Its Applications

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"The Fabric of Farming" will not compete with "Fream" or "Watson and More" as the students' *vade mecum* in his contests with examiners, but it will give him more ideas in 200 pages than many books two or three times as big.

Harwood Long

Blood Groups in Man

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THIS is now the third edition of this book, which has been deservedly popular. Since the last edition many new blood-group antibodies have been discovered which define new blood-group antigens but, on the whole, these have been found to be associated with the known blood-group systems; indeed, the authors state that it looks as if we are nearing the end of the blood-group systems which can be detected by the tests in use up to now, and express the hope that for the future of the science of human genetics, and for the delight of blood groupers, many more systems exist and that their recognition only awaits the devising of suitable tests. This pin-points the importance of the technical advances in this field over the past few years, which have made so much progress possible, and also indicates the predominant interest of these workers in human genetics, which is more evident in this edition than it was in previous ones.

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This book has now become an established favourite and the new edition will be welcomed by all those interested in human blood groups.

F. Stratton

METABOLISM OF NORMAL TISSUES AND NEOPLASMS *IN VITRO*

By Prof. J. H. QUASTEL, F.R.S., and I. J. BICKIS
McGill-Montreal General Hospital Research Institute, 3619 University Street, Montreal

As is well known from the pioneer work of Warburg, characteristic features of tumour metabolism are the high rates of aerobic and anaerobic glycolysis coupled with low respiratory-rates. It is equally well known, however, that a number of normal mammalian tissues show relatively high rates of anaerobic glycolysis, but in these tissues glycolysis is largely abolished under aerobic conditions when oxidative processes are proceeding normally. In most tumours, the high rate of anaerobic glycolysis is reduced possibly to half its value when oxygen is present, but the glycolysis still proceeds at a relatively high rate.

The phenomena of a moderate respiration-rate and high sustained aerobic and anaerobic glycolysis are displayed not only by tumours. Warburg reported that the mammalian retina possesses a high aerobic glycolysis, although he considered that some sort of damage occurs in the preparation or examination of the tissue. A variety of normal tissues possesses characteristics ascribed to tumours. For example, a high aerobic glycolysis obtains in placenta, in kidney medulla and in jejunum mucosa and in the myeloid cells of rabbit bone marrow. It seems, as Greenstein[1] has pointed out, that a *specific* tumour metabolism does not exist, and that no clear-cut distinction can be drawn between normal and tumour tissues on the basis of metabolic criteria. Yet tumours tend to show a fairly uniform metabolism, their chemical activities, however different in origin these tumours may be, resembling each other much more than do those of the many varieties of normal tissues that give rise to tumours. Tumours tend to converge, biochemically speaking, to a uniform type of tissue. Warburg[2] considers that cancer cells originate from normal body cells in two phases, the first phase being an irreversible injuring of respiration and the second being a process whereby some of the injured cells "succeed in replacing the irretrievably lost respiration energy by fermentation energy. Because of the morphological inferiority of fermentation energy the highly differentiated body cells are converted by this into undifferentiated cells that grow wildly—the cancer cells". He points out that the irreversible damage to respiration, which is held to be the first phase of carcinogenesis, "need not be an actual decrease in the respiration quotient but merely an uncoupling of respiration, with undiminished overall oxygen consumption". Warburg lays emphasis not so much on the absolute magnitude of respiratory-rate of tumour cells, but on the relation that obtains between respiratory- and glycolytic-rates. Weinhouse[3] points out that cancer cells show no obvious impairment of respiratory ability, there being "no doubt of the ability of miscellaneous tumours to convert glucose (and fatty acids) to carbon dioxide at rates similar in magnitude to that of non-neoplastic tissues". Moreover, the effects of metabolic inhibitors such as fluoride ion or dinitrophenol are similar in neoplastic and normal tissues[4]. There is no indication from such evidence that oxidative phosphorylation occurs in tumour mitochondria in a manner different from that in the non-cancer material.

It seems to us that more light may be thrown on the general problem of the relation between the aerobic and anaerobic aspects of metabolism, and on the significance of these aspects of metabolism for growth in tumours and normal tissues, by a systematic investigation of the quantitative relations existing between respiration, glycolysis and the rates of biosynthetic processes in these tissues.

If tumours, in spite of their abilities to carry out a variety of oxidations similar to those occurring in normal tissues, cannot utilize aerobic energy as efficiently as normal tissues owing to an uncoupling phenomenon, the ability of tumours to carry out certain biosynthetic changes aerobically should be diminished compared with that of normal tissues. Even the demonstration that tumour mitochondria resemble normal tissue mitochondria in bringing about oxidative phosphorylations does not necessarily imply that in the tumour cell the adenosine triphosphate made available by the mitochondria is as available for cell syntheses as in the normal cell. Only experiments with intact cells will make this information available.

We have, therefore, carried out experiments with tumours and normal tissues in an attempt to discover whether respiratory energy in tumours is less effective than that of normal tissues for biosyntheses, and whether glycolytic energy is superior to that of respiratory energy for such processes in tumour cells.

For the purpose of this investigation, we have concentrated our attention on the quantitative aspects of the incorporation of glycine into proteins of tumour and normal tissues. We have compared the amounts of glycine incorporated under aerobic and anaerobic conditions with the energy released by respiration or by glycolysis in terms of adenosine triphosphate formed by these processes. We have, in fact, compared the 'efficiencies' of a variety of tissues for amino-acid incorporation in terms of available adenosine triphosphate.

Our choice of a system of amino-acid incorporation for the study of energy utilization by tissues under aerobic and anaerobic conditions has been determined by the relatively recent studies that have shown that such incorporation is greatly dependent on the presence of adenosine triphosphate, or on the release of that substance. This incorporation may consist of an exchange reaction or a protein synthesis[7], but presumably a process of peptide-like bond formation occurs which is a system requiring energy[8–12]. It is known[13] that slices of normal rat livers and of hepatomas incorporate ^{14}C-labelled alanine or glycine into the slice proteins, the rate of incorporation into the hepatoma proteins being greater than that into the normal liver proteins. Zamecnik and Keller[14] point out that incorporation of several ^{14}C-labelled amino-acids into proteins takes place anaerobically in a cell-free liver homogenate so long as adenosine triphosphate or a system generating this substance is present. No competition occurs between the various amino-acids tested. Rabinovitz *et al.*[15] have found that incorporation of several labelled amino-acids into proteins of Ehrlich ascites carcinoma cells is

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For the purpose of this investigation, we have concentrated our attention on the quantitative aspects of the incorporation of glycine into proteins of tumour and normal tissues. We have compared the amounts of glycine incorporated under aerobic and anaerobic conditions with the energy released by respiration or by glycolysis in terms of adenosine triphosphate formed by these processes. We have, in fact, compared the 'efficiencies' of a variety of tissues for amino-acid incorporation in terms of available adenosine triphosphate.

Our choice of a system of amino-acid incorporation for the study of energy utilization by tissues under aerobic and anaerobic conditions has been determined by the relatively recent studies that have shown that such incorporation is greatly dependent on the presence of adenosine triphosphate, or on the release of that substance. This incorporation may consist of an exchange reaction or a protein synthesis[7], but presumably a process of peptide-like bond formation occurs which is a system requiring energy[8–12]. It is known[13] that slices of normal rat livers and of hepatomas incorporate ^{14}C-labelled alanine or glycine into the slice proteins, the rate of incorporation into the hepatoma proteins being greater than that into the normal liver proteins. Zamecnik and Keller[14] point out that incorporation of several ^{14}C-labelled amino-acids into proteins takes place anaerobically in a cell-free liver homogenate so long as adenosine triphosphate or a system generating this substance is present. No competition occurs between the various amino-acids tested. Rabinovitz *et al.*[15] have found that incorporation of several labelled amino-acids into proteins of Ehrlich ascites carcinoma cells is

supported anaerobically under conditions of active glycolysis (see also LePage, ref. 26, and Nyhan and Busch, ref. 27). They[15] show that 2,4-dinitrophenol inhibits oxidation-linked incorporation at concentrations at which it stimulates oxygen uptake, but it is a feeble inhibitor of amino-acid incorporation linked with glycolysis. A cell-free system incorporating ^{14}C-amino-acids into proteins has been prepared from Ehrlich ascites carcinoma cells[16], the activation of the amino-acid carboxyl group being also dependent upon adenosine triphosphate[17]. Ziegler and Melchior[18] point out that the amino-acid incorporation system of liver is dependent on integrity of the cell and is very susceptible to lack of oxygen, facts that can be interpreted on the basis of a demand for adenosine triphosphate both for activation and incorporation of amino-acid. A need for glucose for optimal incorporation of amino-acid into a tumour (Gardner lymphosarcoma) was demonstrated by Kit and Greenberg[19], who also found that glycolytic and respiratory inhibitors inhibit the incorporation. The incorporation of labelled glycine and alanine into lymphosarcoma cells was not greater than in the normal spleen cell[19]. Uptake, as well as incorporation, of amino-acids is a process requiring energy. Negelein[20] has demonstrated that uptake of L-histidine into a malignant rat sarcoma takes place anaerobically so long as glucose is present and that glucose stimulates the aerobic uptake.

Attempts have been made by us to answer the following questions:

(1) Is the respiration of tumours 'impaired' in the sense that energy derived from it is less effective in securing amino-acid incorporation into proteins than in normal tissues?

(2) Is the energy utilized for amino-acid incorporation into proteins as effective anaerobically (in the presence of glucose) as that aerobically, either in normal tissues or in tumours?

(3) Do tumours differ from other highly proliferating tissues, including those of the embryo, in the efficiency with which oxidative or glycolytic energy is used for incorporation of amino-acid into protein?

In the following preliminary studies we have investigated the incorporation of glycine-1-^{14}C into the proteins of Ehrlich ascites carcinoma cells and into the proteins of sarcoma 37, Novikoff hepatoma, Walker 256 carcinosarcoma, and a melanoma in *DBA* mice, as well as into the proteins of embryos (chick and rat) and of normal mammalian tissues.

We have calculated the amounts of incorporation in terms of adenosine triphosphate made available by respiration or by glycolysis. It is known[21] that tumour mitochondria give a P/O ratio of approximately 3·0, resembling, in this respect, the mitochondria of normal tissues[22].

We have used for the description of our results a term, which we call, provisionally, the 'efficiency quotient', which is the ratio of amino-acid incorporated into the protein to the adenosine triphosphate theoretically made available by respiration or by glycolysis.

If the respiration is given by the term Qo_2 then, taking P/O = 3·0, the available adenosine triphosphate is given by the term $Q_{ATP} = 6Qo_2$. If the amount of incorporation of amino-acid into tissue protein is given by the term A in counts per minute per milligram dry weight protein, then the efficiency quotient E is equal to $A/6Qo_2$. (The efficiency quotient could be equally well expressed in terms of moles of amino-acid incorporated per mole adenosine triphosphate. A simple calculation, given the experimental conditions, will give this ratio. We prefer the ratio as described, however, for the time being, until it is certain that the label in the protein is due only to the amino-acid incorporated.)

Again, if anaerobic glycolysis is given by the term $Qco_2(N_2)$, then adenosine triphosphate made available by this process will be identical with this term, as a molecule of lactic acid liberated by glycolysis is accompanied by the formation of a molecule of adenosine triphosphate. Therefore, the efficiency quotient, for anaerobic glycolysis, will be given by the term $A'/Qco_2(N_2)$, where A' is the amount of incorporation (counts per minute per mgm. dry weight protein) under anaerobic conditions in presence of glucose.

If respiration is carried out in presence of glucose, the efficiency quotient will be given by the term $A''/\{6Qo_2 + Qco_2(o_2)\}$, where $Qco_2(o_2)$ is the measure of the aerobic glycolysis and where A'' is the amount of amino-acid incorporation (counts per minute per mgm. dry weight protein).

Experimental Methods

All studies were carried out *in vitro* either with slices of tumours that had been grown in mice or rats or in fertile hen's eggs (at the chorio-allantoic membranes), or with washed ascites cells obtained from Swiss white mice after intraperitoneal injection. Ascitic fluid was withdrawn with a hypodermic syringe after six days growth and the cells were separated by centrifuging. They were washed three or four times with 8–10 volumes of cold isotonic saline to remove blood elements and soluble ascitic constituents. Sarcoma 37 was grown in white Swiss mice (subcutaneously or as ascites intraperitoneally) or in chick embryos. Normal tissues came mainly from hooded male rats in the weight-range 200–300 gm. 'Regenerating' liver was produced in these rats by surgical removal of the left and middle lobes. Rat embryos came from pregnant hooded rats and chick embryos came from white Leghorn eggs.

Solid tumours or normal mammalian tissues were cut into slices as soon as possible after killing the animals (by decapitation). The organs or solid tumours were placed in ice-cold Ringer media (without glucose) and tissue slices cut with a Stadie-Riggs slicer. Retina was cut into squares of 0·5 cm.^{2} and incubated as such. Embryos were used whole, with as little damage as possible, after removal of amnion and allantoic membranes. (Under the conditions of our experiments, no visible damage—apart from some change in opacity—took place in the embryos during the incubation procedure.) For each experiment tissue slices from one organ or tumour were used, with the exception of thymus, when slices from the organs of four animals were pooled. Tumours were only chosen that were free from visible necrosis.

The conventional Warburg manometric apparatus was used for estimating respiratory and glycolytic rates for the incubations in which the incorporation of glycine-1-^{14}C into the tissue proteins was measured. The Ringer media used were those described by Umbreit *et al.*[23], the calcium content, however, being 1·5 mM. Glucose, when added, was 10 mM. Glycine-1-^{14}C was diluted with non-radioactive glycine, so that the final concentration used was 2mM. The glycine was placed in the side tube of the Warburg manometer vessel and only tipped into the vessel after appropriate gassing with oxygen, or with

93 per cent oxygen + 7 per cent carbon dioxide or with 93 per cent nitrogen + 7 per cent carbon dioxide and thermal equilibrium at 37°. The total volume of medium in each vessel was 3 ml. In aerobic experiments (with air or oxygen) the centre wells of the manometer vessels contained potash for absorption of carbon dioxide. To ensure complete anaerobiosis, when using nitrogen – carbon dioxide mixtures, sticks of yellow phosphorus were inserted in the centre wells of the manometer vessels.

In all experiments, the results of which are quoted below, the radioactivity of the glycine-1-^{14}C incubated with the tissues was 10^5 counts per min. per vessel, the total incubation period being 1 hr. With ascites cells the incubation period was shortened to 30 min. to prevent too large a fall in *p*H in glucose media.

The wet weights of tissues used in each vessel were quickly obtained with a torsion balance, avoiding all possible injuries especially with retinæ or embryos. Dry weights were established by drying corresponding amounts of tissues, prepared and handled in exactly the same way, at 110° C., to constant weight. The amounts of tissues per vessel were kept approximately the same (80–100 mgm. wet weight).

After incubation, the manometer vessels were placed in crushed ice, their contents (cell and medium) were homogenized in a 'Teflon' pestle homogenizer, precipitated with trichloracetic acid (final concentration 13 per cent) and further homogenized. The precipitates were extracted three times with 6 per cent trichloracetic acid (first extraction carried out for 15 min. at 90° C. to remove nucleic acids), once with ethanol, once with ethanol-ether (3 : 1) at 60° C. for 10 min., once with ether, and finally the residual proteins were suspended in chloroform-ether (4 : 1), plated on aluminium disks and counted. The counting was done with a thin mica end-window Geiger-Müller tube and an automatic scalar and timer. The counted data were corrected for background and self-absorption. The activities given are the actual counts and are expressed as counts per minute per milligram dry weight protein.

Crabtree Effect and Aerobic Glycolysis

The relation between the values for aerobic glycolysis and the diminution of respiratory-rate brought about by the presence of glucose (Crabtree effect) is seen in the results, obtained with tumours, given in Table 1. These results indicate that the ratio of the aerobic glycolysis-rate $Q_{CO_2}(O_2)$ to the difference between the respiratory-rates found in the absence Q_{O_2} and in the presence $Q_{O_2}(G)$ of glucose is constant, approximately 6, with the tumours under investigation.

Such a relationship would be expected if the adenosine triphosphate produced in the tumour cell is approximately constant, whether glucose is present or not, under the experimental conditions. We should then expect that adenosine triphosphate lost by operation of the Crabtree effect is approximately balanced by that gained by aerobic glycolysis. The following relation should hold:

$$\{Q_{O_2} - Q_{O_2(G)}\} \times 2\frac{P}{O} = Q_{CO_2(O_2)}$$

Table 1. Relation between Aerobic Glycolysis and Change of Respiratory-rate due to Glucose Addition

Tumour	Q_{O_2} no glucose added	Q_{O_2} with glucose present $= Q_{O_2(G)}$	$Q_{O_2} - Q_{O_2(G)}$	$Q_{CO_2(O_2)}$	$\frac{Q_{CO_2(O_2)}}{Q_{O_2} - Q_{O_2(G)}}$
Ehrlich ascites (1)	14·0	8·0	6·0	33	5·5
(2)	12·3	7·1	5·2	30	5·7
(3)	12·1	8·1	4·0	29	7·2
(4)	11·3	6·9	4·4	26	5·9
(5)	11·8	6·3	5·5	26	4·7
					Average value = 5·8
Sarcoma 37 (solid, grown in fertile eggs)	9·8	6·6	3·2	20	6·2
Novikoff hepatoma	9·7	7·0	2·7	16·2	6·0
Walker 256 carcinosarcoma	6·8	5·4	1·4	9·0	6·4

The results given in Table 1 indicate that this relationship holds if P/O = 3·0, which is the experimentally observed value obtained with tumour mitochondria. The value of P/O = 3·0, for the intact tumour cell, would indicate that respiration and phosphorylation are tightly coupled in the tumour cells investigated.

The facts obtained are consistent with the view that there is a competition for adenine nucleotides by the hexokinase system in the tumour cell and the respiratory system in the tumour mitochondria[29,30]. Weinhouse[31] has pointed out that tumour mitochondria may be highly permeable to the adenine dinucleotide, diphosphopyridine nucleotide.

Effect of Glucose on the Aerobic Efficiency of Ehrlich Ascites Carcinoma Cells

Typical results showing the aerobic efficiencies of Ehrlich ascites cells for incorporation of amino-acid in the absence and in the presence of glucose are given in Table 2. The results make clear the conclusion that the efficiency with which these tumour cells, under aerobic conditions, incorporate glycine into their proteins as a result of availability of adenosine triphosphate is the same in the absence of glucose as it is in the presence of glucose. It is evident that energy released by tumour respiration, even in the absence of added glucose, is available for amino-acid incorporation, and is quantitatively as effective as the respiratory energy of rat embryonic cells as shown by observations given in Table 3. Moreover, the presence of glucose makes little difference to the efficiency of amino-acid incorporation in spite of the fact that the rate of respiration is considerably

Table 2. Effects of Glucose on Aerobic and Anaerobic Efficiencies of Ehrlich Ascites Carcinoma Cells for Incorporation of Glycine-1-^{14}C into Proteins

	No glucose added	Glucose added 10 m*M*
Aerobic		
Q_{O_2}	12·3	7·1
$Q_{ATP(O_2)} = 6 \times Q_{O_2}$	73·8	42·6
$Q_{CO_2(O_2)}$	—	28·4
$Q_{ATP(O_2)}$ (corrected for aerobic glycolysis) $= 6Q_{O_2} + Q_{CO_2(O_2)}$	—	71·0
A_{O_2} = amino-acid incorporated aerobically into tumour protein (c./min./mgm.)	118	114
Efficiency quotient $= A_{O_2}/Q_{ATP(O_2)}$	1·6	1·6
Anaerobic		
$Q_{CO_2(N_2)}$	2·1	57·3
$Q_{ATP(N_2)} = Q_{CO_2(N_2)}$	2·1	57·3
A_{N_2} = amino-acid incorporated aerobically into tumour protein (c./min./mgm.)	1	115
Efficiency quotient $= A_{N_2}/Q_{ATP(N_2)}$	—	2·0

Table 3. Efficiencies of Aerobic and Anaerobic Incorporation of Glycine-1-^{14}C into Tissue Proteins

	Q_{O_2}(G)	A_{O_2}(G) (c./min./mgm.)	E_{O_2}(G) (corrected for aerobic glycolysis)	$Q_{CO_2}(N_2)$	A_{N_2}(G) (c./min./mgm.)	E_{N_2}(G)
Ehrlich ascites carcinoma	7·1	137	1·9	62	138	2·2
Sarcoma 37 (subcut. from mice)	5·0	60	1·5	19·7	24	1·2
Sarcoma 37 (from fertile eggs)	6·6	105	1·8	40·1	89	2·2
Sarcoma 37 ascites (from mice)	4·5	97	2·0	44	102	2·3
Novikoff hepatoma	7·0	80	1·5	33·6	68	2·0
Walker 256 carcinosarcoma	5·4	70	1·6	27·2	55	2·0
Melanoma *S.*91 (*DBA* mice)	4·6	45	1·3	16·7	25	1·5
Embryo, rat	10·9	121	1·7	21·8	29	1·3
Embryo, chick (6th day)	14·8	221	2·5	30·6	45	1·6
Rat liver	6·5	33	0·8	2·2	0·0	0·0
Rat 'regenerating' liver (42nd hr. after operation)	10·0	85	1·4	3·2	0·0	0·0
Rat kidney cortex	22·2	9	0·07	2·8	0·0	0·0
Rat kidney medulla	19·9	20	0·17	11·5	1	0·09
Rat spleen	6·3	48	1·2	4·6	2	0·4
Rat thymus	8·8	81	1·5	12·7	9	0·7
Rat lung	4·7	31	1·1	5·7	2	0·3
Pigeon pancreas	4·8	42	1·4	4·6	1·0	0·2
Rat brain	12·8	2	0·03	9·4	0·0	0·0
Cattle retina	10·2	4	0·07	32·4	3	0·1

reduced by the presence of glucose. (Calculation shows that only a very small fraction of the available adenosine triphosphate is used for incorporation of glycine, approximately one mole of glycine being incorporated for 500 moles adenosine triphosphate released.) The diminution of available adenosine triphosphate by the reduction of respiratory-rate is apparently almost exactly balanced by that released by aerobic glycolysis. This phenomenon applies to other experimental tumours that we have examined and would seem to indicate that the high rate of aerobic glycolysis characteristic of tumours is a means whereby the tumour cell may retain its content of available adenosine triphosphate undiminished in spite of the operation of the Crabtree effect.

It has been shown by Emmelot and van Vals[24] that the incorporation of DL-glutamate-1-^{14}C and DL-leucine-1-^{14}C into the proteins of mammary carcinoma ascites cells is essentially the same whether glucose is present or not, a fact in agreement with the results given in Table 2. On the other hand, Davis and Busch[25] point out that the specific activities of proteins of slices of Walker 256 carcinosarcoma, following aerobic incubation with L-aspartate, L-glutamate, or L-alanine, all uniformly labelled with carbon-14, are markedly enhanced by the presence of glucose. It is possible that this result is partly (if not wholly) due to the aerobic conversion of each of these amino-acids, which are uniformly labelled, in presence of glucose, to a mixture of radioactive amino-acids the incorporations of which are independent of each other[14]. Nyhan and Busch[26] have indicated that L-glutamate is metabolized by Walker 256 carcinosarcoma *in vivo*.

Effect of Glucose on the Anaerobic Efficiency of Ehrlich Ascites Carcinoma Cells

Glucose is essential for anaerobic incorporation of amino-acids into Ehrlich ascites carcinoma cells. Results given in Table 2 show that the efficiency of glycolytic energy for incorporation of glycine into the tumour proteins is a little higher than that of respiratory energy. Both quotients, however, for the tumours investigated are of the same order of magnitude, and a variety of experiments have shown that the aerobic and anaerobic efficiency quotients for incorporation of amino-acid into proteins of the tumours investigated are approximately equal.

The aerobic efficiency is calculated on the basis of P/O = 3·0 for tumour respiration. If the value of P/O is significantly less than this, the 'efficiency' of respiratory energy for incorporation of amino-acid would increase and exceed that of glycolytic energy. It is evident that the results do not support the hypothesis that there is any 'impairment' of tumour respiration in the sense that respiratory energy is uncoupled from phosphorylation and, therefore, from an accompanying biosynthetic process such as incorporation of amino-acid.

It would be expected that the efficiency of adenosine triphosphate for incorporation of amino-acid into the tumour proteins would be independent of its source of production, either respiration or glycolysis. The fact that experiments with intact tumour cells show that aerobic and anaerobic efficiency quotients are approximately equal is good support for the conclusion that adenosine triphosphate is the main energy factor in the process of incorporation of amino-acid into the proteins of intact cells.

Efficiencies of Aerobic and Anaerobic Incorporation of Glycine into Tissue Proteins by Tumours and by Normal Mammalian Cells and Embryos

Results of a survey of the relative rates of incorporation of glycine-1-^{14}C into the proteins of tissues under aerobic and anaerobic conditions, glucose being present under both conditions, are given in Table 3. They demonstrate the following facts:

(1) The highest efficiency ratios, both aerobic and anaerobic, occur with the most rapidly proliferating tissues, namely, tumours and embryos. Thymus and pancreas, among normal mammalian tissues, have the highest aerobic efficiencies—possibly this is connected with their high capabilities for synthesizing proteins.

(2) The ratio of anaerobic efficiency to aerobic efficiency approximates to unity with tumours, the anaerobic value being usually a little higher than the aerobic. With adult tissues, the aerobic efficiency term usually exceeds the anaerobic value; this occurs also with embryos.

(3) Adult normal tissues having high respiratory activities (for example, kidney cortex, brain cortex) may have low aerobic efficiencies for the incorporation of glycine into their proteins. Similarly, normal tissues having relatively high anaerobic glycolytic activities (for example, kidney medulla, retina) may have little or no anaerobic efficiencies for the incorporation of glycine into their proteins.

Table 4. Relative Efficiencies of Ehrlich Ascites Carcinoma and Chick Embryo for Incorporation of Glycine and L-Phenylalanine into Proteins

		EO_2(G)	EN_2(G)
Glycine-1-^{14}C	Ehrlich ascites carcinoma	1·6	1·7
	Chick embryo	2·4	1·5
Phenylalanine-2-^{14}C	Ehrlich ascites carcinoma	1·3	1·2
	Chick embryo	1·5	0·9

It should be pointed out that the aerobic efficiency quotients for incorporation of amino-acid into the proteins of adult tissues and embryos will need a small correction for the amount of radioactive protein produced from the radioactive carbon dioxide formed by oxidation of the amino-acid. This is largest with kidney cortex, but is negligible with Ehrlich ascites carcinoma cells and sarcoma 37, or thymus, which bring about very little oxidation of glycine to carbon dioxide. Chick embryo, rat brain cortex and rat liver have about one-sixth of the oxidizing power of rat kidney cortex on glycine for equivalent weights of tissue.

The results given in Table 3 make clear the fact that in the development of embryo into adult organs a marked drop takes place in the ability of the tissue to incorporate glycine into its proteins, although aerobically, and anaerobically with certain tissues (kidney medulla, brain cortex, retina), energy is apparently available in amounts comparable with those in the embryo. Further investigations are needed to establish the reasons for the diminished efficiency of adult tissue for incorporation of amino-acid into their proteins; but one possible cause may be the development in the adult tissues of new enzyme systems dependent upon adenosine triphosphate, which are needed for the special functions of these tissues. Less adenosine triphosphate, therefore, is available for their amino-acid incorporating systems. The relative lack of the ability to incorporate amino-acid observed in adult tissues such as kidney or brain cortex, under anaerobic conditions in presence of glucose, points to the fact that in such tissues cytoplasmic, or extra-mitochondrial, adenosine triphosphate (made available by anaerobic glycolysis) cannot be used for incorporation of amino-acid. This stands in contrast to the effects of embryonic (or tumour) glycolysis. Further investigations, based on these conclusions, may help to throw light on the nature of the specific biochemical changes that arise with differentiation and growth of the embryo into the adult stage.

Observations recorded in Table 4 show that the conclusions concerning the incorporation of glycine into the proteins of Ehrlich ascites carcinoma cells and chick embryo apply also to L-phenylalanine, which was used under exactly the same experimental conditions as glycine. Investigations with other amino-acids are proceeding.

Tumour and Embryo

Tumours and embryos, which resemble each other in having relatively high efficiencies for the aerobic and anaerobic incorporation of amino-acid, show, so far as their metabolism is concerned, a number of significant differences. For example:

(1) The presence of glucose, which depresses respiratory activity of tumours, has but little effect on that of rat or chick embryo.

(2) The presence of malonate greatly depresses both the rate of respiration and the rate of incorporation of amino-acid into proteins of Ehrlich ascites carcinoma cells and of chick embryo in the absence of glucose. These results, which are shown in Table 5, are consistent with the fact that the citric-acid cycle operates in these tissues[31]. When glucose is added, however, malonate exercises relatively little effect on the rate of respiration of the tumour, or on the rate of incorporation of glycine, in contrast to its large effect on embryo metabolism in the presence of the sugar. It appears, therefore, that in the presence of the respiratory poison, which blocks incorporation of amino-acid into tumour protein, glucose reverses the effect—a phenomenon not seen with embryo. In this respect embryo resembles adult tissue more than it resembles tumour. Nyhan and Busch[27] have shown that malonate suppresses the specific activity of proteins of Walker 256 carcinosarcoma exposed aerobically to labelled glutamate, and that the addition of glucose alleviates this suppression.

The reversing effect of glucose on the depressing effect of metabolic inhibitors on the amino-acid incorporation of tumours is also seen in its action on Ehrlich ascites carcinoma cells in presence of bilirubin or 2 : 4 dinitrophenol. Representative results obtained with ascites cells, given in Table 6, indicate that bilirubin can act as an uncoupler of phosphorylation from oxidations, a conclusion already reached by Zetterström and Ernster[32] in their studies of the effects of bilirubin on liver mitochondria. The substance, at low concentrations, suppresses incorporation of glycine into the tumour proteins, an effect partially reversed by the addition of glucose. It abolishes the Crabtree effect, and resembles in these respects the action of 2 : 4-dinitrophenol (Table 6). It might be noted in this connexion that sarcomycin, an inhibitor of growth of Ehrlich ascites carcinoma, inhibits glycine incorporation into ascites proteins[33]. The inhibition is diminished by the presence of glucose, but not as much as when bilirubin or dinitrophenol is the inhibiting agent.

Table 5. Effects of Sodium Malonate on Metabolism of Ehrlich Ascites Carcinoma and of Chick Embryo (5 days old)

	QO_2		Glycine-1-^{14}C incorporation into proteins (c./min./mgm.)	
	Ascites	Embryo	Ascites	Embryo
No glucose present				
Without malonate	10·9	8·0	144	159
With malonate 20 m*M*	4·8	5·6	12	69
With glucose present				
Without malonate	5·3	7·8	138	172
With malonate 20 m*M*	4·3	4·8	119	60

Table 6. Effects of Bilirubin and Dinitrophenol on Metabolism of Ehrlich Ascites Carcinoma

	QO_2	Glycine-1-^{14}C incorporated in proteins (c./min./mgm.)
No glucose present		
Control	13·0	83
With bilirubin		
0·16 m*M*	8·8	13
0·32 m*M*	1·3	4
With 2 : 4-dinitrophenol		
0·1 m*M*	15·5	36
Glucose (10 m*M*) present		
Control	6·5	110
With bilirubin		
0·16 m*M*	10·3	51
0·32 m*M*	10·8	52
With 2 : 4-dinitrophenol	15·3	87

The fact that the addition of glucose may restore to the tumour, in the presence of inhibitors of oxidative phosphorylation, its ability to incorporate amino-acids in its proteins, emphasizes the biological importance of this sugar in enabling the tumour cell to withstand the toxic effects of certain metabolic poisons.

Summary

(1) The ratio of rate of aerobic glycolysis ($Q_{CO_2(O_2)}$) to the difference between the respiratory-rates found in the absence (Q_{O_2}) and in the presence ($Q_{O_2(G)}$) of glucose is constant, approximately 6, with the experimental tumours investigated (Ehrlich ascites carcinoma cells, sarcoma 37, Novikoff hepatoma and a melanoma). This relationship is expected if the adenosine triphosphate produced in the tumour cell is approximately constant, whether glucose is present or not, the adenosine triphosphate lost by the Crabtree effect being balanced by that gained by aerobic glycolysis. The facts indicate that the following relation holds:

$$\{Q_{O_2} - Q_{O_2(G)}\} \times 2\frac{P}{O} = Q_{CO_2(O_2)}$$

where P/O = 3, and that, therefore, respiration and phosphorylation in the intact tumour cell are tightly coupled.

(2) The efficiency with which the tumour cells under investigation incorporate glycine, under aerobic conditions, into their proteins as a result of availability of adenosine triphosphate is the same in the absence of glucose as it is in the presence of glucose. The efficiency term is defined by the ratio A/Q_{ATP}, where A is the radioactive amino-acid incorporated into the tumour protein (given in counts per minute per milligram dry-weight protein) and Q_{ATP} is the available adenosine triphosphate calculated as equal to $6Q_{O_2}$ under aerobic conditions in the absence of glucose or to $6Q_{O_2} + Q_{CO_2(O_2)}$ in the presence of glucose, or to $Q_{CO_2(N_2)}$ anaerobically in the presence of glucose. The energy released by tumour respiration, in the absence of glucose, calculated on the basis of P/O = 3 for the tumour cell, is quantitatively as effective for amino-acid incorporation into tissue proteins as the respiratory energy of rat embryo.

(3) The efficiency of glycolytic energy for incorporation of amino-acid into tumour proteins is approximately equal to, though usually a little higher than, that of respiratory energy.

(4) A survey of the relative rates of incorporation of glycine-1-^{14}C into the proteins of a variety of tissues under aerobic and anaerobic conditions, glucose being present, shows: (*a*) The highest efficiency ratios, both aerobic and anaerobic, occur with the most rapidly proliferating tissues, namely, tumours and embryos. Thymus and pancreas, among normal mammalian tissues, have the highest aerobic efficiencies. (*b*) The ratio of anaerobic efficiency to aerobic efficiency approximates to unity with tumours, the anaerobic value being usually a little higher than the aerobic. With adult rat tissues, and with chick and rat embryos, the aerobic efficiency term usually exceeds the anaerobic value. (*c*) A number of normal tissues (for example, kidney medulla, retina), having relatively high anaerobic glycolytic rates, have little or no anaerobic efficiencies for the incorporation of glycine into their proteins. It would seem that extra mitochondrial, or cytoplasmic, adenosine triphosphate (made available by glycolysis) cannot be used in these tissues for amino-acid incorporation. This is to be contrasted with the efficiency of embryonic energy made available by anaerobic glycolysis. Many adult mammalian tissues show relatively low aerobic efficiency terms for amino-acid incorporation, as compared with those of tumour or embryo.

(5) Essentially similar results are obtained with tumour and embryo using phenylalanine-2-^{14}C instead of glycine-1-^{14}C.

(6) Tumours and embryos, which resemble each other in their high aerobic and anaerobic efficiency terms for amino-acid incorporation into their proteins, differ in their relative responses to the effects of malonate in the absence and presence of glucose. Although malonate greatly inhibits both respiration and incorporation of amino-acid into proteins of Ehrlich ascites cells and of chick embryos, the addition of glucose almost eliminates the malonate inhibition with the tumour cells, but has little or no effect on the malonate inhibition with chick embryo.

(7) Bilirubin, at low concentrations, suppresses incorporation of glycine into Ehrlich ascites cells. It acts as an uncoupling agent, and, like dinitrophenol, abolishes the Crabtree effect. Its inhibitory effect on amino-acid incorporation is partially reversed by glucose, which may play an important part in enabling a tumour cell to withstand the toxic effects of certain metabolic poisons.

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THE FISH CULTURE RESEARCH STATION, MALACCA

By Dr. C. F. HICKLING, C.M.G.
Acting Director

FISH culture has the same object as agriculture and stock breeding, namely, to increase by all possible means the production of food far above the level which would be produced naturally. Like agriculture, fish culture includes the elimination of unwanted plants and animals, their replacement by desirable species, the improvement of these species by cross-breeding and selection, and the improvement of the substratum by the use of fertilizers.

The remarkable rate of increase of human populations is bringing increasing pressure on food production. In Malaya, for example, the population will, on present trends, double every twenty years. It is widely realized that every possible piece of suitable land must be brought into production, and great efforts are being made to do so. No less must every suitable piece of water produce the maximum weight of fish.

But whereas fundamental research in tropical agriculture does not lack research stations of the first magnitude, there has so far been lacking a research station, on the scale of a major agricultural station, for the fundamental study of fish culture. The Fish Culture Research Station at Batu Berendam, Malacca, was designed to fulfil this need. Its function may be briefly described as to give a new look to an ancient and stereotyped traditional technique.

The Station was built at a cost of some £235,000 to Colonial Development and Welfare Funds, and was officially opened on August 21, 1957, ten days before Malaya attained independence. The Station is intended to serve, in the first instance, all tropical Commonwealth territories; and so the problems it will tackle will be such as are common to all, or most of, these territories. It is intended to accept contributions from all such territories as are willing to support this work.

Batu Berendam is nearly five miles north-west of Malacca, on first-class tarmac roads; and the Station has municipal piped water and electricity. Besides the laboratory building, there are six houses for senior staff adjacent, and fourteen quarters for junior staff. The pond area is only some 400 yd. from the laboratory.

The Station is at approximately sea-level; there is no seasonal variation in either temperature or rainfall. Rainfall averages about 100 in. a year; air temperature varies but little from a diurnal range of 76–86° F.

The laboratory (Fig. 1) is a one-story building of modern design, with six working rooms in addition to offices, workshops and stores. There is research accommodation for eight scientists; as the establishment of the Station is for a director and five scientific officers, there is accommodation for visiting workers, who will be very welcome. The laboratory has been designed for extension as required.

Novel features of the laboratory are, first, an underground rain-water tank, from which rain-water collected from the roof is automatically pumped to a cistern at the top of the tower seen in Fig. 1, to descend by gravity through polyethylene piping to thirty-six concrete tanks, in which fish can be held for quarantine or examination, and in which they can be bred. Secondly, eight similar concrete tanks are in a blacked-out compartment with powerful 'daylight' lighting. An automatic time-switch enables fish or plants in this compartment to be subjected to whatever number of hours of 'daylight' an experiment requires. Interesting results might come from subjecting tropical fish, which know no seasonal variation in their length of day, to temperate lighting conditions.

The estate in which the fish-ponds are constructed is 170 acres of former swamp land; of these, 82 acres are actual water surface. There are 164 ponds, of a size ranging from breeding ponds of 1/100 acre, to ponds of 4½ acres. A unique feature of the Station is that, within each group, the ponds are identical in shape, area and depth. Fig. 2 shows some of the ponds.

The pond site is mainly composed of a heavy lateritic clay. This is an infertile acid soil with impeded drainage, which supports crop plants with difficulty, even after the addition of fertilizers. That part of the estate which has not been used for the construction of ponds is still largely swamp land and supports a stand of 'gelam' trees. These trees are known to grow well on land which proves sterile and often toxic when afterwards prepared for agricultural use. The harmful nature of such soils may be caused by a high concentration of soluble sulphates and aluminium salts which have been found to be present[1]. Seepage water from swamp land adjacent to the fish-ponds was analysed, and contained more than 0·1 per cent of sulphate and more than 6 p.p.m. of aluminium. This affects the pond water-supply, which is taken from the Malacca River (*p*H 6·0, sulphate negligible). After flowing through the canal, water taken at the pump house, which supplies the ponds, has a *p*H of 3·5 and contains more than 60 p.p.m. of sulphate.

Similar toxic effects, resulting from the drainage of swamp land, have been frequently reported (for example, in Uganda[2]). In spite of considerable agricultural research, the problem of using such land profitably remains largely unsolved. By using such land for fish culture, therefore, the Fish Culture Research Station at Malacca is doing pioneer work. Already it has been found that heavy crops of fish, including the Chinese grass carp, can be carried, apparently in perfect health and with rapid growth, in ponds constructed in 'gelam' soil. The effect of lime dosages will be given below.

The natural production of wild fish in such poor conditions is very low. Nine of the ponds belonging to the Station, which had had standing water in them for at least two years and still had the original

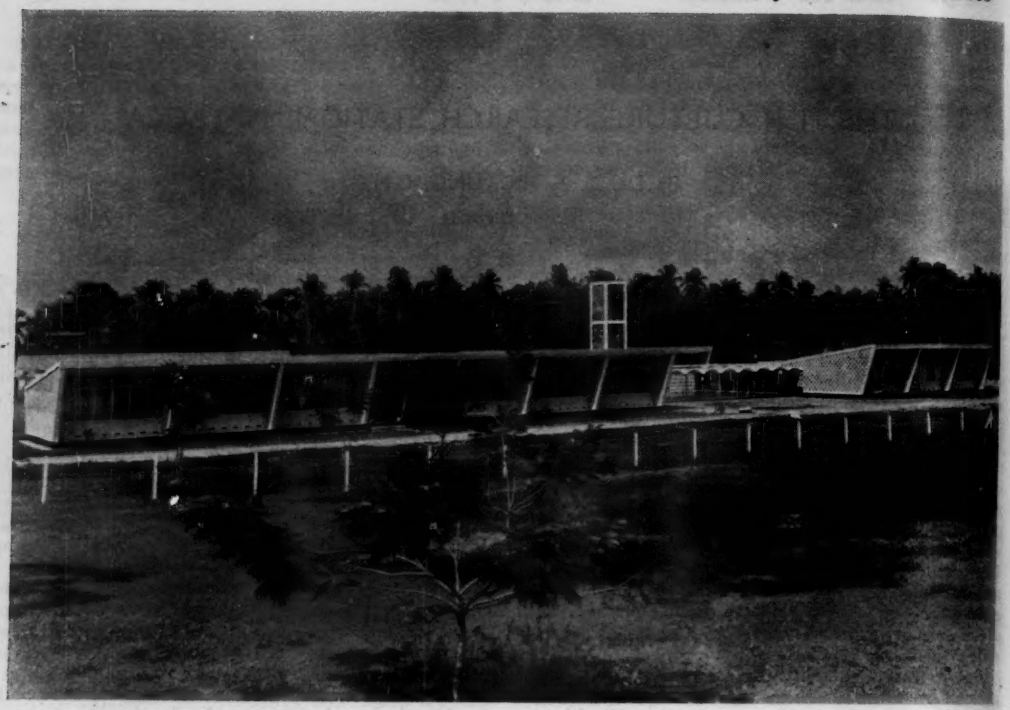

Fig. 1. The Fish Culture Research Station, Malacca

swamp-fish fauna, were cleared by the use of a suitable fish toxin, and the total fish population weighed. The average standing crop was 18 lb. per acre, comparable with the annual production of the fresh-waters of England and Wales, of Lake Tiberias in Palestine, and of Lake Mendota in Wisconsin[3].

By clearing away swamp vegetation, liming to neutrality, and stocking with desirable species, already yields at a rate of more than 300 lb. per acre per annum have been obtained, without the use of fertilizers, in these ponds.

Naturally, the work of the first year has been chiefly concerned with clearing the swamp vegetation from the ponds, and breeding stocks of fish to put in them. Nevertheless, a full-scale experiment has been carried out, in the thirty-six 1-acre ponds, on the effect of six levels of lime dosage each replicated six times, from zero to 2 tons per acre. In Fig. 3 is shown the effect on the *p*H of these successive doses. Each point is the mean for six ponds. It will be seen that the untreated controls had a *p*H of 5, and that the *p*H rapidly increased with increasing lime dosage until, after about 15 cwt. limestone per acre, the *p*H levelled out at about neutrality.

This quantity, namely, 15 cwt. limestone per acre, appears to be much less than that quoted by the text-books. Most authors follow Schäperclaus, who himself copied agricultural tables by Trenel. From Schäperclaus's table, heavy loams or clays, such as exist at the Station, of a *p*H of 4·5–5, would need 25 cwt. lime per acre, or say 50 cwt. chalk. The table published by Macan, Mortimer and Worthington[5] would prescribe 18–36 cwt. limestone per acre. However, these experiments at Malacca are the first, so far as I know, to be done on the large scale in conditions of very acid soil and water.

This work is continuing with an investigation of the effect on fish production of graduated doses of phosphate and potash, with and without limestone. The final object of this line of work is to increase the fish crop with the least expense, by using the best ascertained fertilizer mixture.

The changes in vegetation consequent on the clearing of the swamp vegetation, flooding with water, and the application of lime in various doses, have provided an interesting study.

From typical swamp consisting mainly of sedges, flooding alone tends to eliminate only short plants such as *Eriocaulon* and *Xyris*. The addition of lime, as well, suppresses the growth of *Rhynchospora aurea*, *Scleria poaeformis* and *Philydrum lanuginosum*, leaving *Fimbristylis acuminata* and *Isachne globosa* comparatively unaffected. These latter disappear only with regular cutting. As a result of liming, the typical lime-lover *Chara flexilis*, originally not present in the area, appeared and spread, especially after the draining and refilling of the ponds. The disappearance of emergent vegetation was followed by an increase in planktonic algae, except in those ponds where there were dense beds of *Chara*. No doubt, in these latter, intense competition for available salts suppresses the growth of the algae. Ponds with unstable bottoms and muddy water differed in that both submerged macrophytes and planktonic algae tended to be suppressed, probably due to the extreme turbidity of water. There is some evidence that in limed ponds, submerged macrophytes, particularly *Chara*, give way eventually to beds of *Enhydrias angustipetala*, and this process is hastened by the application of artificial fertilizers such as potassium chloride – ammonium phosphate mixture, which is being tried out.

Fig. 2. Fish ponds at the Fish Culture Research Station, Malacca

As yet, a study of fodders, to supplement the food naturally produced for the fish in the ponds, has not passed beyond a preliminary study of the digestive enzymes of fish, and of that part of the natural food which is utilized by the fish. But undoubtedly this work will lead on to a study of cheap fodders, chiefly oil-cake residues, and finding out what part of these can be utilized. The final object of this line of work would be something approaching a balanced ration, taking into account not only the added fodder, but also the natural food supply. This has been done for fish-ponds in temperate climates, but not under tropical conditions.

The Station holds stocks of the following species: *Tilapia zillii* from East Africa; *T. mossambica*, local; *Tilapia* sp. from Zanzibar and hybrids of these *Tilapia* species; *Cyprinus carpio*, common carp, local; *Puntius javanicus*, supplied by the Fisheries Department of the Federation of Malaya; *Catla catla*, from Calcutta; *Osphronemus olfax*, the giant gouramy, local; *Trichogaster pectoralis*, Sepat Siam, local; *Ctenopharyngodon idellus*, the Chinese grass carp, from China.

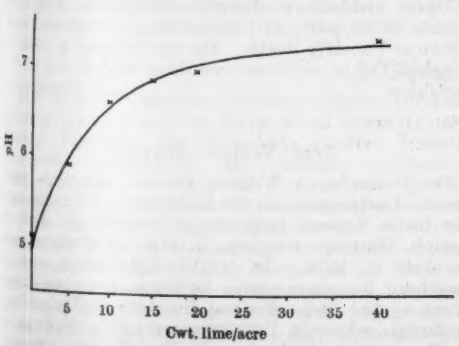

Fig. 3. Effect of lime on pH. Approximate conditions after 100 days

Stocks of the last-named fish are being held against the visit of Dr. H. D. Slack, who, on the basis of work now being carried out by him in the Department of Zoology, University of Glasgow, supported out of Colonial Development and Welfare Funds and by the Nuffield Foundation, hopes to induce them to spawn in captivity. This valuable fish has never spawned in fish-ponds; the fry have to be imported, chiefly from Canton.

Chief interest at present centres on the *Tilapia* hybrids. The Malayan stock of *Tilapia mossambica* is very inbred, and the cross with new stock from East Africa not only has, as might be expected, great vigour and strength; but also certain crosses give a very skew sex-ratio, indeed up to 100 per cent males; and a marking experiment just ended indicates that the cross-bred fish may grow twice as fast as either the Malayan or the African parent stocks. An extremely promising field is open here; for a fish of very rapid growth, but which cannot breed in the rearing ponds, comes very close to the ideal pond-fish for commercial or subsistence raising.

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OBITUARIES

Prof. Niels Bjerrum

We regret to record the recent death of Niels Bjerrum. He was born in Copenhagen on March 11, 1879, and studied in the University there, taking the degree of Dr. Phil. in 1908 and presenting a dissertation on the hydrolysis of chromium compounds. This work was extended by the discovery of the third isomeric hexahydrate of chromic chloride and, as a result of a study of the absorption spectra of solutions of chromic salts, by the provision of an experimental foundation for the hypothesis of the complete dissociation of strong electrolytes in solution, which Bjerrum in 1909 was the first to make really significant.

Bjerrum later published many valuable researches in various branches of electrochemistry, the theory of acids and bases, the measurement of hydrogen ion concentrations, amphoteric electrolytes, and the behaviour of indicators. He published a monograph on acid-alkali titration and discovered a simple method of eliminating diffusion potentials in measurements of hydrogen ion concentrations. His work in this field had a very significant influence on the development of the modern theory of electrolytes. He developed a simple equation connecting the activity and osmotic coefficients of an electrolyte, and another one relating the activity coefficients to reaction velocity. He worked with Perrin in 1911 on the Brownian movement and later published work on colloid chemistry. In 1923, he published an important paper on the relation of the stages of ionization of polybasic acids to the molecular structure. In 1911, he related the specific heats of steam and carbon dioxide to band spectra. In 1912, he published work with Nernst on the specific heats of steam and carbon dioxide at high temperatures as determined by the explosion method, and in 1914 he laid the foundations of the theory of the infra-red spectra of polyatomic molecules by introducing the so-called valency force-field, which has led to all subsequent developments in this subject. In these publications he applied the newly developed quantum theory of specific heats to gases.

In 1914, Bjerrum became professor of chemistry in the Royal Danish Veterinary and Agricultural College in Copenhagen, an institution which has had a distinguished history. Since then he has published a large amount of important work in various branches of inorganic and physical chemistry and on problems in agricultural chemistry, notably on the factors which determine the *p*H of soils and their fertility. He wrote an excellent small text-book on inorganic chemistry which has gone through several editions, including two in an English translation.

In his prime, Niels Bjerrum was a tall man of handsome presence with an open and friendly expression, and a calm dignity of manner. He will be greatly missed by his many friends in all parts of the world.

J. R. Partington

Prof. D. C. Grahame

David Caldwell Grahame, professor of chemistry at Amherst College, Amherst, Massachusetts, U.S.A., died suddenly as a result of a coronary thrombosis in London on December 11. He was spending the academic year as a visiting professor in the University of Bristol.

Prof. Grahame was born in St. Paul, Minnesota, on April 21, 1912. He received a bachelor's degree in chemical engineering at the University of Minnesota in 1935 and a Ph.D. degree in physical chemistry at the University of California at Berkeley in 1937. From then until 1939 he was an instructor in chemistry at Berkeley. From 1939 he was on the staff at Amherst College, becoming assistant professor in 1942, associate professor in 1947 and professor in 1953. He received an honorary M.A. from Amherst in 1953. In 1945 he was a member of the staff of the Radiation Laboratory, Massachusetts Institute of Technology. He taught at Harvard in the summer of 1948. He had visited Europe on several occasions since 1952, having been invited to present papers at meetings of the Deutsche Bunsengesellschaft (1955) and the Chemistry Section of the Soviet Academy of Sciences (1956). He held a Guggenheim Fellowship during his stay in Bristol.

His scientific work was largely in the field of electrochemistry, particularly in the study of the electrical double layer located at the interface between a metal and an electrolytic solution. He developed an accurate method of determining the properties of this double layer by measuring its electrical capacity with an alternating current bridge using a growing drop of mercury or gallium as electrode. With this elegant technique he obtained precise results for an extensive variety of electrolytes. Starting from the basic thermodynamic principles, he showed how these results could lead to accurate values of the ionic concentrations at the surface of the electrode and hence to a reliable picture of the structure of the interfacial region. He gave a lucid and comprehensive account of the theory of this procedure in *Chemical Reviews* in 1947, which was further developed in his paper with Barbara Soderberg in the *Journal of Chemical Physics* in 1954.

More recently, Prof. Grahame had turned to the adsorption of poly-electrolytes and the use of non-aqueous solutions. In recent months he had devoted much attention to a study of the distribution of potential in the region within a few Ångströms of the surface of the metal. The progress he made in elucidating the latter was only possible on the basis of the precise experimental work in which he excelled, together with the depth of his insight into the theoretical aspects of the problem. It is regrettable that this work remains incomplete.

David Grahame's cheerful friendliness won him friends in all parts of the world, all of whom must grieve at his early death. He was married in 1935 to Virginia Dakin, who survives him, with a son and a daughter.

R. Parsons

Prof. William Brunner

On December 1 William Brunner, emeritus professor of astronomy in the University of Zurich and the Swiss Federal Institute of Technology, died in Zurich. Brunner was born in Wattwil (Switzerland) on July 8, 1878. In 1900—after having been a merchant for some years—he began studying astronomy at the Swiss Federal Institute of Technology in Zurich, where in 1904 he acquired the diploma in mathematics and physics. During the next twenty-two years he was a teacher in a girls' college, until in

1926, in succession to Prof. A. Wolfer, he was appointed director of the Swiss Federal Observatory and at the same time professor of astronomy in the Institute and in the University of Zurich; both directorship and professorship he occupied until, for reasons of health, he retired in 1945.

Besides lecturing, Brunner carried on the traditional work of the Swiss Federal Observatory, namely, the observation of sunspots. In thirty numbers of the *Astronomische Mitteilungen* and in two volumes of the *Publikationen der Eidgen. Sternwarte* he published the yearly reports on solar activity, which contain above all the relative sunspot numbers as a continuation of the series reaching back to the year 1749, but also articles on the development of sunspot groups and on the prominence statistics. In 1928 the International Astronomical Union handed over to him the publication of the *Quarterly Bulletin on Solar Activity*. During the years 1938–45 he was a member of the executive committee of the International Astronomical Union.

Brunner wrote several popular books, among them "Die Welt der Sterne" and "Pioniere der Weltallforschung". On his retirement in 1945 he was elected an Associate of the Royal Astronomical Society of London.

M. WALDMEIER

NEWS and VIEWS

Forestry Division of the Food and Agriculture Organization: M. Leloup

M. MARCEL LELOUP, director of the Forestry Division of the Food and Agriculture Organization of the United Nations since its creation twelve years ago, is retiring. M. Leloup was a veteran of the First World War and a recipient of the Légion d'Honneur after losing an arm during the battle of Verdun. Having graduated from the famous École Polytechnique, he began his forestry career with the Colonial service of the French Department of Waters and Forests in French Equatorial Africa. In 1932 he returned to Paris to the general direction of the Department of Waters and Forests. In 1936 he was assigned to the Presidence du Conseil and charged with several special missions concerning agricultural and administrative matters. It was in this capacity that he collaborated in the establishment of the Statute of Public Function and was nominated to the highest French administrative body, the Council of State. After the Second World War, M. Leloup became director-general of the Department of Waters and Forests and a Councillor of State. Shortly afterwards he was a member of the French delegation to the Quebec conference of 1945, at which the Food and Agriculture Organization was founded. In May 1946 he became the first director of its Forestry Division. The wide organizational work which he accomplished in this capacity gave him an exceptional knowledge of the problems and possibilities of forests in the world, and he was active in the formation of the Regional Forestry Commission for Europe (1947), for Latin America (1948), for Asia and the Far East (1949) and for the Near East (1953).

E. Glesinger

MR. EGON GLESINGER, formerly deputy director, became director of the Division of Forestry and Forest Products, the new title of the Forestry Division, on January 1. Mr. Glesinger was born in 1907 and was educated in Austria and Czechoslovakia. He graduated in commercial science at Geneva in 1927, obtained a doctorate of law at Prague in 1929 and a doctorate in political science at Geneva in 1932. He also studied at the Institute for International Studies at Geneva, and his thesis on European forests was afterwards published in book form. During 1933–38, Mr. Glesinger was director of the International Timber Committee, an organ of the League of Nations situated first in Vienna and later in Brussels. After the Second World War, he served on the Interim Commission which preceded the formal establishment of the Food and Agriculture Organization, and has been an official of the Organization since its foundation. Besides his post as deputy director of the Forestry Commission, he is at present director of the Timber Division of the United Nations Economic Commission for Europe.

Canadian National Aeronautical Establishment

THE creation of a new Division of the National Research Council of Canada, to be known as the National Aeronautical Establishment, has recently been announced. The formation of the National Aeronautical Establishment was initially authorized in 1951, when it consisted of the aeronautical research facilities of the Division of Mechanical Engineering in the National Research Council, but the heavy and increasing demands on the Establishment have rendered it administratively expedient to separate it from the present Division of Mechanical Engineering, and embody it in a separate Division. The resources of the new Division will consist of the Flight Research Hangar and laboratories at Uplands Airport, the new 5-ft. supersonic wind-tunnel now being constructed at Uplands Airport, and the Aerodynamics and Structures Laboratories in the Montreal Road Laboratories of the National Research Council. The new Establishment will work on defence problems in aeronautical science, and will also be responsible for meeting the aerodynamic and structural research needs of civil aviation and of the aircraft industry.

Mr. F. R. Thurston

MR. FRANK R. THURSTON, head of the Structures Laboratory of the Division of Mechanical Engineering, who has been appointed acting director of the National Aeronautical Establishment, was educated in the United Kingdom, graduating from the University of London. He went to the National Research Council in 1947 from the National Physical Laboratory, Teddington, where he worked on supersonic aerodynamics and on the structural design of aircraft. In the National Research Council he has been responsible for the structural proving of prototype Canadian-built aircraft, and for a wide range of research on structural fatigue, towers and shell structures, and aircraft hydraulic equipment. Representing Canada, Mr. Thurston is chairman of the

Structures and Materials Panel of the Advisory Group for Aeronautical Research and Development of the North Atlantic Treaty Organization and works as a co-ordinator for the Commonwealth Advisory Aeronautical Research Council.

Royal Geographical Society: Medals and Awards, 1959

H.M. The Queen has approved the award of the Royal Medals of the Royal Geographical Society, as follows: *Patron's Medal:* Commander W. R. Anderson, U.S.N., for the first trans-polar submarine voyage, in command of U.S.S. *Nautilus*; *Founder's Medal:* Sir Raymond Priestley, British observer, U.S. Antarctic Expedition, 1958–59, for services to Antarctic exploration.

The Council of the Royal Geographical Society has made the following awards: *Victoria Medal:* Mr. Gerald Seligman, president of British Glaciological Society, for contributions to glaciological research; *Murchison Grant:* Prof. S. P. Chatterjee, professor of geography, Calcutta, for important work organizing and directing the "National Atlas of India"; *Back Grant:* Mr. David Stratton, deputy-leader, Commonwealth Trans-Antarctic Expedition; *Cuthbert Peek Grant:* Mr. W. George Lowe, for contributions to Himalayan and Antarctic exploration; *Gill Memorial:* Mr. G. S. Holland, chief draughtsman, Royal Geographical Society, for contributions to cartography, especially of southern Arabia; *Mrs. Patrick Ness Award* (jointly): Mr. J. H. Miller, deputy-leader, New Zealand party, Commonwealth Trans-Antarctic Expedition, for contributions to Antarctic exploration, and Dr. George W. Marsh, Commonwealth Trans-Antarctic Expedition and Falkland Islands Dependencies Survey, for contributions to Antarctic exploration.

British National Committee on Antarctic Research

The British National Committee on Antarctic Research has been set up to co-ordinate British Antarctic activities in relation to the large scientific programme at present being considered by the Special Committee on Antarctic Research of the International Council of Scientific Unions. This programme includes meteorology, ionospheric studies, auroral physics, geomagnetism, cosmic rays, geology, glaciology, geomorphology, cartography, seismology, gravity, vulcanology, oceanography, sea ice, biology including marine aspects and physiology. The members of the new National Committee are: *Chairman*, Sir Raymond Priestley; Royal Society: Prof. W. V. D. Hodge, Sir Lindor Brown, Dr. H. G. Thornton, Mr. A. H. Sheffield, Prof. F. W. Shotton, Prof. J. E. Smith, Dr. R. L. Smith-Rose, Dr. R. Stoneley and Sir James Wordie; Sir Vivian Fuchs, scientific director, Falkland Islands Dependencies Survey; Brig. M. Hotine, director, Overseas Surveys; Rear-Admiral K. St. B. Collins, hydrographer of the Navy; Dr. G. E. R. Deacon, director, National Institute of Oceanography; Sir Graham Sutton, director-general, Meteorological Office; Dr. G. de Q. Robin, United Kingdom delegate to the Special Committee of Antarctic Research; Sir Miles Clifford, Royal Geographical Society; Dr. E. Hindle, Royal Geographical Society; Mr. G. Seligman, British Glaciological Society; Dr. S. Evans, Scott Polar Research Institute; Mr. J. Paton, Royal Society of Edinburgh; Dr. L. G. C. E. Pugh, Medical Research Council; and Mr. H. H. Lamb, Royal Meteorological Society.

Safety and the Nuclear Power Industry

The hazards which nuclear power stations present were raised by Mr. G. Roberts in an adjournment debate in the House of Commons on January 21. In reply, Sir Ian Horobin, Parliamentary Secretary to the Ministry of Power, said that on the best evidence available there is no danger of radioactive strontium from nuclear power stations in areas of high rainfall like north-west Wales adversely affecting the water supply. Further, although the amount of radioactive strontium descending from the stratosphere is increasing at the present rate, it will be several generations before the equilibrium-level built up in the bones of the population reaches the amber band. The increase in leukæmia began a generation before the first nuclear tests, and there are at present no statistically significant differences, either by area or in time, in the incidence of this disease; specifically there is no statistical difference between north Wales and other parts of the United Kingdom. In the normal operation of a nuclear power station certain radioactive substances might go up the stack but their life is so short that they can be ignored, as can seepage. As regards the release of coolants, monitoring is done with such extreme care that hazard is unlikely. The Wigner type of release which led to the Windscale accident will not be necessary in the new stations where the coolant, carbon dioxide, will operate in a closed circuit. Sir Ian thought that only in very exceptional circumstances could an accident lead to serious contamination and assured the House that Britain is in the forefront not only of constructional ingenuity but also in safety devices in this field.

Glass in Nuclear Research

Important developments in nuclear research in recent years have meant an increased demand for viewing windows to permit clear observation of operations or objects within the bounds of the 'hot' cell walls, and also to facilitate the remote handling of radioactive materials, while providing protection for the operatives. Just as ingenious mechanisms have been evolved for remote handling, so have special types of glass been produced for shielding windows. The Chance-Pilkington Optical Works, at St. Asaph, North Wales, has now developed radiation-absorbing glasses of larger dimensions than have been available hitherto in Europe. Particularly at very short wave-lengths, the attenuation offered by protective walling is proportional to the density of the material employed. The Chance-Pilkington shielding glasses range in density from 2·5 gm. per c.c. to 6·1 gm. per c.c., and allow the design of windows to be, in many cases, of equal thickness to the surrounding wall, thereby reducing the complexity of additive shielding and obtaining the maximum optical advantage from the window.

Upon receipt of large doses of short wave-length radiation, glasses of normal composition tend to discolour to a deep yellow or brown. To overcome this, Chance-Pilkington manufacture a series of glasses which are stabilized. These glasses have amended compositions having the effect of slightly decreasing the transmission of the glass in the unirradiated state, but reducing the tendency for further discoloration under conditions of radiation. Shielding glasses of density 2·5 gm. per c.c. and 4·3 gm. per c.c. are manufactured in polished plate form and also in block form up to 4 ft. 6 in. by 3 ft. in area and 10 in. in thickness, with the limitation

for the time being that no single block shall weigh more than 2,000 lb.

New Process of Making Plate Glass

PILKINGTON BROTHERS, LTD., of St. Helens, Lancashire, have just announced the successful development of a new process of making plate glass. In the new process the plate glass is produced with a fire-polished finish, so that the lengthy grinding and polishing operations which are necessary with the present method of producing plate glass are eliminated. The crux of the process which contains the revolutionary idea is that the molten ribbon of glass, which flows from the usual tank furnace containing the molten glass through a pair of rollers, is then supported on the surface of a bath of liquid metal. This is at a sufficiently high temperature for the under-surface of the glass to take up the extreme flatness of the surface of the liquid metal while at the same time the top surface can be subjected to sufficient heat to produce a good fire polish. This new operation takes place in a chamber containing a controlled atmosphere. Pilkingtons have named the new process the 'float process' and are referring to the new product as 'float glass'. Ordinary window glass, known in the industry as sheet glass, is also produced with a fire-polished surface by drawing upwards from the surface of a bath of molten glass, but the quality of this type of glass is not sufficiently good for its use in motor-car windows, shop windows, or for the preparation of mirrors, and it is for applications of the kind just mentioned that plate glass is in demand. The growth of the motor-car industry has, of course, made the demand for plate glass rise steeply, and this invention is thus a very timely one.

The history of the manufacture of plate glass can be seen to follow a pattern in recent years of the gradual development of an existing process until a fundamentally new idea occurs. About the beginning of the first century A.D., flat glass was first made by the crown process, in which a flat disk of glass was spun on the end of an iron. The method of making sheet glass by fabricating a cylinder which was then split and flattened was first described in about A.D. 1100; the casting and rolling process of making plate glass was invented in France towards the end of the seventeenth century. In 1910 the cylinder method was mechanized and cylinders up to 40 ft. long and 6 ft. in diameter were made. Mechanical production of sheet glass by drawing flat sheets of glass directly from a tank was first effected in Belgium just before the outbreak of the First World War, and in 1920 the continuous flow process of plate glass manufacture was developed in the United States. Pilkingtons have largely been concerned in the development of this process, leading to the famous twin-grinding machine, which grinds and polishes both sides of the glass simultaneously. In the light of this background, it can be seen that this new invention is justifiably described as revolutionary.

Cambridge Instrument Co., Ltd.: New Research Laboratory

CONSTRUCTION has commenced of the new Research Laboratory for the Cambridge Instrument Co. Overlooking the River Cam and Jesus Green, and within ten minutes walk of the centre of Cambridge, the 20,000 sq. ft. building will provide an imposing frontage for the Cambridge factory. The top floor with generous roof lighting will form an ideal design and drawing office, and the three floors below are being fitted with the latest equipment for instrument work involving physics, chemistry, electronics and precision mechanical engineering. The new building heralds a considerable expansion in research, development and design for which staff are now being recruited. The architects are Messrs. Edward D. Mills and Partners, designers of the British Industries Pavilion at the Brussels Universal and International Exhibition, 1958, and Messrs. J. Jarvis and Co. are the main contractors. The new Laboratory is scheduled for completion in June.

Iron and Steel at the Science Museum

A NEW exhibition on the principles underlying the manufacture of iron and steel will be opened by Lord Mills, Minister of Power, on February 6 at the Science Museum, South Kensington. Exhibits have been drawn from the Museum's own collections and contributed by makers and users of iron and steel, by other museums and individual donors, to illustrate the development of that industry from its earliest beginnings. By far the largest contribution has been from the British Iron and Steel Federation, which has not only provided a large number of new exhibits but also financed the whole project. Only outstandingly important technological aspects and developments can be touched upon in a limited space—direct reduction of iron ore, charcoal-blast pig-iron and its fining to malleable iron, coke-iron, foundry work, puddling, crucible steel and cementation, modern blast-furnace practice, Bessemer, open hearth and alloy-steel making and modern methods of casting, forging, rolling, drawing, together with theoretical metallurgy, testing and research. The treatment is not exhaustive but provides an excellent stimulus to further study.

British Association: Granada Lectures

A NEW series of annual lectures, organized by the British Association for the Advancement of Science and sponsored by Granada TV Network, Ltd., on the theme of "Communication in the Modern World", has been announced. The broad purpose of these lectures is to explore the impact of communications in the field of the mass media—Press, radio, television and film—in their sociological, political and technical aspects. The first series of three lectures will be given in London in October 1959: the first by Sir Edward Appleton, who will speak on the significance for society of long-range communication and the exploration of space; the second by Dr. Edward R. Murrow, the American television and radio commentator and news analyst, who will speak on the impact of television and radio in the field of politics and public affairs in a democracy; the third by Sir Eric Ashby, whose subject will be methods of presenting scientific information to the man in the street and the extent to which the part played by the mass media in this field might be extended or improved. The lectures will be delivered to an invited audience, to include representatives of science, industry, politics, education, local government, the mass media and others. There will also be a limited number of tickets available to the public. The texts of the lectures will afterwards be published. With each lecture will be linked a television programme, in which it is hoped the lecturer will participate, dealing with the subject-matter of the lecture in appropriate television form for a wider audience.

Synthetic Quartz Crystals

The Western Electric Company and the Bell Telephone Laboratories in New York have jointly succeeded in growing large crystals of artificial quartz by a process which they hope to develop on a technical scale. It has long been known that small quartz crystals can be developed in silicate solutions by suitable treatment, but in the new process crystals up to 5–6 in. long and 2–3 in. cross-section have been obtained. The method consists in filling a vertical autoclave with an alkaline solution, usually of sodium hydroxide. Small pieces of natural quartz are placed in the bottom to serve as material and high-quality sand will, it is expected, be used in future. Seed plates cut from natural or artificial quartz crystals are hung from a rack in the upper part of the vessel. After sealing, the autoclave is heated to the required temperature and maintained with a constant temperature differential from bottom to top during the processing time, which has varied from a week to several weeks. Growth occurs primarily in one dimension and flat plates are used, which become thicker but change little in other dimensions. The nutrient material dissolves in the hotter lower region and is carried by convection currents to the cooler upper region, where the solution becomes supersaturated and the dissolved silica is deposited in the form of a single crystal. The combination of conditions of high temperature and pressure in the autoclave is severe and the effective sealing of the autoclave has presented a number of problems in the selection of material and design. The crystals produced have no foreign inclusions, can be obtained without optical or electrical twinning, and can be sawn in the most efficient manner.

The Night Sky in February

New moon occurs on Feb. 7d. 19h. 22m. U.T., and full moon on Feb. 23d. 08h. 54m. The following conjunctions with the Moon take place: Feb. 2d. 01h., Jupiter 3° S.; Feb. 4d. 12h., Saturn 4° S.; Feb. 16h. 05m., Mars 6° N. Mercury is too close to the Sun for observation. Venus is an evening star, visible in the south-west after sunset. It sets at 18h. 30m., 19h. 10m. and 19h. 55m. on February 1, 15 and 28, respectively; its stellar magnitude is − 3·3. Its distance decreases during the month from 146 to 137 million miles, and the visible portion of the apparent disk decreases from 0·945 to 0·900. Mars is moving eastwards among the stars and is in Taurus during February. It sets at 2h. 45m., 2h. 25m. and 2h. 05m. at the beginning, middle and end of the month, respectively. Its stellar magnitude decreases from + 0·3 to + 0·8, its distance from the Earth increasing from 94 to 119 million miles. Mars is well placed for observation during the evening. Jupiter rises at 2h. 45m., 2h. 00m. and 1h. 10m. on February 1, 15 and 28, respectively; it is in Libra. The stellar magnitude of Jupiter is − 1·6; conditions for observation are not very favourable. Saturn is a morning star in Sagittarius, rising at 5h. 35m., 4h. 45m. and 3h. 55m. at the beginning, middle and end of the month, respectively. It is unfavourably placed for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Feb. 17d. 21h. 43·6m., 115 Tau. (*D*); Feb. 18d. 18h. 20·9m., 124 H^1 Ori. (*D*); Feb. 20d. 0h. 23·0m., λ Gem. (*D*); Feb. 21d. 19h. 14·2m., α Cnc. (*D*). *D* refers to disappearance.

Announcements

Sir John Cockcroft has accepted the invitation of his fellow Trustees of the future Churchill College, Cambridge, to become its first Master. Future Masters of the College will be appointed by the Crown on the advice of the Prime Minister.

Life peerages (baronies) have been conferred on the following: Sir Eric James, high master of Manchester Grammar School; Sir Edwin Plowden, chairman of the United Kingdom Atomic Energy Authority; Prof. Lionel Robbins, professor of economics in the University of London; and Sir Hartley Shawcross, the distinguished lawyer.

Prof. R. G. Baskett has been appointed director of the National Institute for Research in Dairying, University of Reading, as from August 1, 1959.

Dr. T. S. England has been appointed head of airborne radar at the Royal Radar Establishment, Malvern, as from January 1.

The New York Academy of Medicine has awarded its Medal to Dr. Peyton Rous for his contributions to knowledge of neoplastic disease. The Academy has awarded its Plaque to Mr. Montgomery B. Angell, for outstanding service to the New York Academy of Medicine as its legal adviser.

The first of the new afternoon lectures for sixth-form children from schools in London and the Home Counties were delivered at the Royal Institution, Albemarle Street, London, W.1, on January 27 and 28 by Mr. E. Jenkins (Atomic Energy Research Establishment, Harwell), who spoke on "Radioactive Elements". This lecture is to be repeated on February 3 and 4. The programme of lectures for the remainder of the term is as follows: "The Origin of Man", by Prof. J. Z. Young, on February 10, 11, 17 and 18; "The Significance of Colour in Inorganic Chemistry", by Prof. R. S. Nyholm, on February 24 and 25 and March 3 and 4 (sponsored by the Salters' Company); "Electrons in Solids", by Prof. R. King, on March 10, 11, 17 and 18 (sponsored by the Bell Telephone Laboratories).

The American Society of Mechanical Engineers, in co-operation with Purdue University Thermophysical Properties Research Center, is organizing a symposium on "The Thermal Properties of Gases, Liquids and Solids", to be held at Purdue University, Lafayette, Indiana, during February 23–26. Further information can be obtained from Prof. R. A. Olsen, School of Mechanical Engineering, Purdue University.

The Seventh Latin American Congress of Chemistry will be held in Mexico City during March 29–April 3. On the afternoons of April 1 and 2 an international symposium will be held on "Recent Progress in Organic Chemistry". The morning sessions during these two days will be devoted to about twenty shorter papers dealing with steroids and related natural products. Further information can be obtained from Dr. Alberto Sandoval, Instituto de Quimica, Ciudad Universitaria, Mexico 20, D.F.

Erratum. Dr. S. Bricteux-Grégoire states that there was a mistake in the manuscript entitled "Utilization of Formate for the Biosynthesis of Glycine Carbon-1 and -2 in *Bombyx mori*", published in *Nature* of November 29, p. 1515. In Table 1, for "C-1, 20,300 and C-2, 43,500", read "C-1, 43,500 and C-2, 20,300".

ZOOLOGY IN NIGERIA

ECOLOGY OF LAGOS LAGOON

THE University College of Nigeria has achieved a standing among the university institutions of the Commonwealth which few even among the most visionary spirits would have dared to hope for at the time of its foundation little more than ten years ago in a forest clearing at Ibadan. Students recruited from a country where there are few secondary schools have been enabled to pursue courses for honours degrees in a wide range of subjects and disciplines within the Faculties of Arts, Science, Agriculture and Medicine, and the standards achieved by them are those which the University of London, with which the College is in special relationship, requires of its honours students. That the College should have produced a body of graduates ready to take their places as administrators and teachers in a country soon to achieve independence is a tribute alike to the innate abilities of the Nigerian student and to the sound and imaginative teaching of the professors and lecturers who, over the years, have staffed the several departments of the College. Nor in these formative years has the prosecution of research been neglected and there has recently been published, in the *Philosophical Transactions of the Royal Society*, a series of papers* by Prof. J. E. Webb (two of them jointly with Dr. M. B. Hill), of the Department of Zoology, which will exemplify the kind of work which is being done and which, while born of and descriptive of West African conditions, probes deeply and with discernment into problems of fundamental and more general biological interest.

The papers are concerned primarily with the life-history and ecological requirements of the Nigerian lancelet, *Branchiostoma nigeriense* Webb, one of the many species contained within the group of the Cephalochordata the structure and development of which show them to stand very close to the ancestral stock of the vertebrates. The observations range in their scope from considerations of physical geography and coastal morphology to an analysis of the significance to animals of the size and shape of sand grains, without, however, ever losing its essential unitary character as an ecological investigation.

Lagoon systems occur on the Guinea Coast, within the general areas of the Ivory Coast, Togoland and Dahomey–Western Nigeria. They are formed behind barrier beaches of sand, the sand drifting from west to east along the beaches and offshore under the action of obliquely breaking waves developed under the predominantly south-westerly winds. Lagoons are formed by the seaward advance of the barrier beach or by enclosure by outwardly growing spits curved at the tips to form offshore bars. The lagoons may remain filled with water by virtue of river drainage or access to the sea. There is, however, a tendency for them gradually to fill with sediment, first to become swamps and then dry land. Aerial photographs show that the cycle has been repeated many (about twenty) times in the 25,000 years of the post-glacial history of the Guinea Coast. Narrow bands of sparse vegetation alternating with broader zones of more luxuriant growth mark, respectively, the lines of former sand barriers and of lagoon deposits.

The Lagos lagoon system is open to the sea by narrow channels and receives the drainage water from some 40,000 square miles of forest country. In the drier months from December to March higher salinities rising from about 10 to 30 per mille prevail, whereas with the coming of the rains the salinity falls sharply to give brackish or fresh conditions which tend to persist with brief intermissions from about April to December. It is against this background of changing salinities that the appearances and disappearances of *Branchiostoma* within the waters and bottom deposits of the lagoon must be viewed.

Laboratory experiments show that the lancelets tolerate changes in temperature greater than those normally encountered within the lagoon but that they die in less than a day's immersion in water of less than 13 per mille salinity. They are, in fact, absent from the lagoon from about mid-May to mid-December, when the salinity is 13 per mille. By mid-December conditions are again favourable for restocking of the lagoon from the persistent populations of lancelets of the outside waters. These latter were found to spawn from about August to October and to give rise to pelagic larvæ ready to seek the bottom and to metamorphose at the earliest some eleven weeks after fertilization, a tally being kept of the length of the larvæ and of the number of their gill pouches at the different ages. Larval life can be extended to twenty weeks or so if a suitable substratum is not available for settlement. Re-population of suitable lagoon deposits can then begin at the moment that the rise in salinity of the lagoon water permits of survival of larvæ drifted in from the sea. Once settlement has been achieved, the lancelets remain in the deposits until killed off in May by the fall in salinity following upon the onset of the March rains. In the meantime, a few of the resident population will have spawned: the young are, however, short-lived, being killed by the influx of fresh water while still in the pelagic phase or shortly after metamorphosis.

The various aspects of these successive events are examined in detail. None is of more interest than the observations and experiments which bear on the substratum requirements of the metamorphosing and adult lancelets. In Nature, animals only occur in coarse sand containing 25 per cent of particles smaller than 0·2 mm. in diameter and in which the finer silts bulk less than 1·5 per cent. While, in laboratory tests, a few animals would settle in soils containing rather more of the fine sand or of the silt, they did not long remain viable. Moreover, it was only in the coarse, open sands that they burrowed normally; in mixtures of progressively finer particles they remained

* *Phil. Trans. Roy. Soc.*, B, **241** (1958). The Ecology of Lagos Lagoon: I, J. E. Webb, The Lagoons of the Guinea Coast, pp. 307–318; II, M. B. Hill and J. E. Webb, The Topography and Physical Conditions of Lagos Harbour and Lagos Lagoon, pp. 319–334; III, J. E. Webb, The Life History of *Branchiostoma nigeriense* Webb, pp. 335–353; IV, M. B. Hill and J. E. Webb, On the Reactions of *Branchiostoma nigeriense* Webb to its Environment, pp. 355–391; V, J. E. Webb, Some Physical Properties of Lagoon Deposits, pp. 393–419.

partially buried or rested on the surface. But particle-size is not all. Smooth-grained mixtures are sought after, sharp particles avoided; again, sands with silt or organic matter in excess of a certain amount are not favoured, but neither are those which lack micro-organisms on the surfaces of the grains. It is shown that the particular size-range of particle mixtures chosen by the lancelets are those with a high porosity, that is to say, they have a system of canal spaces within which water may circulate freely.

Thus, in the event, the lancelets select substrata by their texture, but benefit by other properties dependent on texture, such as free drainage.

The community concept, in so far as it applies to animals living in the deposits of the ocean floor, has long been known to be founded upon the selection by animals of soils of varying texture and particle size. Few investigations in recent years have been more revealing of the bases of this selection than those described in the last of the five papers here reviewed.

J. E. Smith

IMMUNOLOGICAL ASPECTS OF ALLERGIC DISORDERS

"Immunological Aspects of Allergic Disorders" was the subject of the symposium of the autumn meeting of the British Society for Immunology, held jointly with the British Association of Allergists, at the Wellcome Building, London, on November 14.

A. W. Frankland introduced the subject as a clinician engaged in the treatment of 'allergic' disorders, for which antigen – antibody reactions are considered responsible. Seasonal pollen asthma, the least complicated allergic disease, responds equally well in 90 per cent of subjects to pre-seasonal treatment with a high dosage of an extract of the pollen of a single grass as with extracts of mixed grass, illustrating the need for studies of pollen antigens. A beneficial effect, however, is also found in between one-third and one-half of the patients treated with saline injections.

The significance of reactions to skin tests showing the presence of reaginic antibodies capable of sensitizing the skin requires clinical confirmation. In 50 per cent of subjects sensitive to pollen skin tests, observed over a period of ten years, clinical sensitivity does not appear to become important. Inhalation tests may confirm the presence of clinical sensitivity.

The clear description of allergic reactions is desirable. Immediate reactions are seen in pollen sensitivity; delayed reactions are of the tuberculin type. An unexplained relationship between these is present in reactions to insect bites. The first bites are usually innocuous, but after a time they may excite reactions of the delayed type. At a later stage, reactions of the immediate type appear, and still later, in some cases, there may be a complete absence of reaction to the bites.

R. Augustin, in a paper entitled "Allergens and other Pollen Antigens", reported her investigations into the relationship of grass pollen antigens, which excite in rabbits the production of precipitating antibodies, and those grass pollen antigens, which are allergenic for man, reacting with the reaginic but non-precipitating antibodies which sensitize the skin, as shown by reactions to skin tests. The complicated pattern of antigen – antibody precipitation lines produced in agar–gel diffusion tests with rabbit antisera were studied for their relevance to allergenic activity in skin tests on man. Enzymic and other studies showed that the antigens of timothy and cocksfoot pollen that were most highly electrically charged were not significantly related to skin-test allergens. The main allergens were found in those antigens having the electrophoretic mobilities and salting out properties of human serum α- and β-globulins. These antigens, on one hand, were precipitated with rabbit antiserum and, on the other, accounted for almost all the allergenic activity in man. The concentrated fractions obtained in this way contained 10–100 million Noon units/mgm. (1 mgm. pollen equals 1,000 Noon units by definition). Coupled preparations of this active allergenic material with red cell stromata and hide powder (Baird and Tatlock) were effective in removing, specifically, the reaginic antibodies from the serum, as shown by passive transfer tests, thus providing a method for demonstrating the presence of reagins.

J. G. Feinberg and H. Grayson discussed the results of "A New Look at the Antibodies to so-called 'Common' Antigens of Grass Pollens". Using antibody gradients and eccentric patterns of precipitation, they showed that rabbit antibodies to pollens have a greater affinity for homospecific than for heterospecific pollens. A similar but greater homospecificity is shown with rabbit antisera to ovalbumin and ovalbumin tagged by means of arsenilic acid. It was suggested that pollen treatment should be based on homospecific pollen allergens.

Some observations on the antibodies related to ragweed pollen were discussed by B. Rose, who with Sehon and his colleagues have found that there are four antibodies in the serum of sensitive patients. Erythrocytes coated with antigen provided a highly sensitive and specific method for demonstrating non-precipitating antibodies. Two of the antibodies found were skin-sensitizing, one was a blocking antibody, and one a hæmagglutinating antibody. The blocking antibody was found in the slow-moving γ_2-globulin and the skin-sensitizing antibody in the fast-moving γ_1-globulin. The skin-sensitizing antibody also moved with the fast-sedimenting *S*19 fraction. The hæmagglutinating antibodies are considered to be similar to the ordinary divalent antibodies and were found in all serum fractions containing skin-sensitizing and/or blocking antibodies.

M. Loveless discussed the reproducibility and practical value of the conjunctival test for estimating the degree of hypersensitivity to ragweed pollen. Reactions to increasing concentrations were observed, and a good correlation was found between the degree of hypersensitivity and the titre of blocking antibody present in the serum after treatment by injection. The conjunctival test was used as a guide to the safe dosage of pollen for injection in a 'repository' form, in emulsion of liquid paraffin and 'Arlacel'. Such a guide is necessary since the dosage of pollen in the limited number of injections of repository required is far higher than the initial doses of aqueous extracts used for conventional methods of pollen treatment.

Investigations into allergic hypersensitivity in man to *Aspergillus fumigatus* were described by J. Pepys, R. W. Riddell, K. M. Citron, Y. M. Clayton and E. I. Short. Allergic reactions to the ubiquitous and perennial spores of *A. fumigatus* consist of rhinitis, conjunctivitis and asthma. The fungus also causes respiratory infections, associated with pulmonary eosinophilia, consisting of transient, recurrent pulmonary infiltrations, febrile episodes and eosinophilia of the sputum and blood. *A. fumigatus* is found with a significantly greater statistical frequency in the sputum of asthmatics without pulmonary eosinophilia than in the sputum of patients with other respiratory disorders.

Hypersensitivity as the cause of the pulmonary eosinophilia was investigated. Skin-prick tests with extracts of *A. fumigatus*, *flavus*, *nidulans*, *niger* and *terreus* gave positive reactions to almost all species in nearly every hypersensitive subject. Culture-medium filtrates, one month old, were the most potent allergens, followed by the cell sap of fresh mycelia, and then by conventional carbol saline extracts of dried mycelium.

Prick tests produced immediate weal-reactions, followed after 3–12 hr. in a number of cases by a 'late' reaction. This reaction was occasionally quite extensive and differed macroscopically and in its more rapid appearance and resolution from the delayed tuberculin type of reaction. Histologically it showed early, intense and persistent infiltration of eosinophile cells, as compared with the perivascular infiltration of round cells in the tuberculin reaction. These features and the demonstration of precipitating antibodies in the serum of patients by means of concentrated extracts of *Aspergillus*, especially *A. fumigatus*, suggest that the 'late' reaction is of the Arthus type.

Bronchial reactions to inhalation tests with extracts of *Aspergillus* were most evident in patients with pulmonary eosinophilia. Proof that the fungus plays a part in the clinical picture must rest, however, not only on evidence of its presence in the sputum, but also on immunological confirmation.

Preliminary investigations into the immunochemistry of *Cladosporium* were reported by J. Longbottom, B. Hayward and R. Augustin. Extracts of spores and mycelium were found to be equal in potency in skin tests. Their active components were found, as with pollen, to be non-dialysable and to be associated with the protein-like material which did not move from the base-line in paper chromatography.

At least eight antigens were demonstrable in agar-gel tests with rabbit antisera to *C. fulvum* and *C. herbarum*. Antiserum to *C. herbarum* gave precipitation lines with the antigens of *C. fulvum*, but not *vice versa*. The *Cladosporium* antigens have the mobilities of serum albumins and α- and β-globulins. Of four antigens shown by immunoelectrophoresis in *C. fulvum*, three were found to be non-allergenic in man. Patients tested with the extracts and with proteolytic digests of the two species showed individual variations, as might be expected.

J. F. Ackroyd discussed a patient with allergic thrombocytopenic purpura due to antazoline. Antazoline added to the serum caused lysis of the platelets and the formation of a precipitate, both mediated by the same antibody. Taking the drug on two later occasions resulted in a rise in the fallen antibody titre. Formation of a precipitate at these times was not, as it had been previously, accompanied by complement fixation; and no cause could be found for this.

The observation that the formation of a precipitate with complement fixation was produced by adding the drug to serum, collected while the patient was taking sufficient antazoline to give high enough blood-levels to excite thrombocytopenia, suggests that the union of the drug and the platelets is very labile. This dissociability may explain why so few persons develop purpura on taking antazoline and other drugs such as sedormid, quinine and quinidine, for which a similar lability has been observed.

J. Pepys

THE NEW FERODO RESEARCH LABORATORIES

By Dr. R. C. PARKER
Research Director

On November 21, some sixty-one years after Mr. Herbert Frood, the founder of Ferodo Ltd., produced his first friction material, H.R.H. the Duke of Edinburgh formally opened the new Research Laboratories of the firm by unveiling a suitably inscribed bronze plaque. Work on current problems was afterwards inspected. In the following week a series of open days were arranged, one of which was reserved for the teaching staffs of universities, local technical colleges and schools and for the staffs of research institutes.

The building (Fig. 1) is in the form of a splayed *E* having a total floor area of 75,000 sq. ft. The upper two stories of the front of the building house the Administration Department, a Plant Development Department and offices for senior managers, while the two-storied centre wing with a floor area of 12,000 sq. ft. houses the Physical, Chemical and Metallurgical Laboratories. All the internal partitions in both laboratories and offices are built on a five-foot module and can be readily mounted or removed as desired. The left-hand wing, covering 21,000 sq. ft., comprises an Engineering Test House, a workshop and a test car garage, and the right-hand wing, with an area of 18,000 sq. ft., contains pilot plant. The site of the building is such that when further space is required the length of the three projecting wings can be increased by 50 per cent. The building is pleasantly situated on the main road from Chapel-en-le-Frith to Hayfield, and is set among landscape gardens.

The main heating of the offices and the laboratories is by warm water that circulates through special steel coils embedded in the ceilings. Supplementing

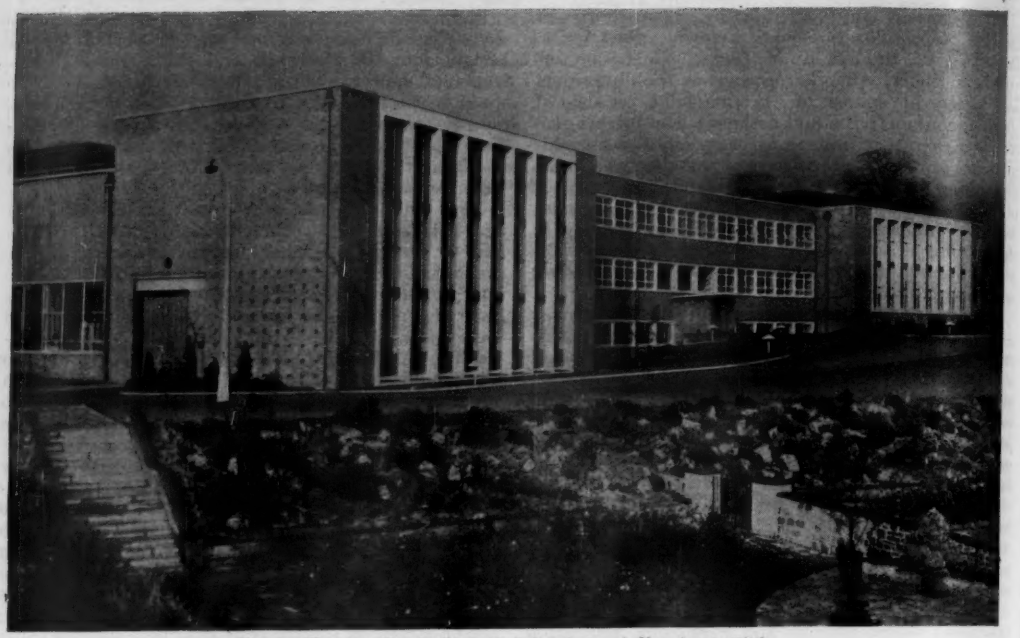

Fig. 1. The new Ferodo Research Laboratories at Chapel-en-le-Frith

this is warm filtered air which is led into each five-foot module, and afterwards extraction is by a fan system so designed that the air in each room is changed from four to five times every hour. The two outer wings are heated by unit heaters of the down-stream type fixed high in the roof and controlled by thermostats fixed at working level. Ventilation of these outer wings is by means of fresh-air ducts connected to the unit heaters and by extraction fans in the roof. In addition to gas, hot and cold water is provided, together with the usual electrical services. Steam is provided at 180 lb./sq. in. and at 40 lb./sq. in., and compressed air at 100 lb./sq. in. All services to the laboratories are housed in timber ducts that run along the walls from window-sill-level to floor-level. Services to island benches are accommodated beneath the timber and reinforced concrete floor.

The laboratories are lit by 80-watt fluorescent tubes in fittings on the ceilings, giving an average value of illumination of about 30 lumens per sq. ft. at desk-level. The Test House and Experimental Production Laboratory are lit by 250-watt mercury vapour lamps and 500-watt tungsten filament lamps which give an illumination of some 18 lumens per sq. ft.

The research and development programmes carried out in the new building are all directed towards the production of improved types of friction material for the automotive industry and for railways, aviation, mining and indeed every industry that employs moving machinery. Almost irrespective of the application, demands on friction materials are ever increasing both in number and intensity, and in the case of automotive brakes, for example, the peak energy dissipation per unit area has more than doubled within the past decade. Attention has not only to be given to the level of friction under varying conditions of pressure, speed, temperature and surface roughness, but also to durability, squeal, oil resistance, water resistance and many other such factors.

In order to be able to produce durable material with the required value of friction, research in the laboratories is carried out along many inter-related channels. Long-term work is carried out on the mechanism of friction and wear, and this involves the study of the bulk and surface properties of a wide range of different materials. Next, basic work of a medium-term nature follows on the friction materials themselves, and this has, as one of its objects, the further study of certain aspects of behaviour to which the long-term programme has directed attention. The third, last and most important link in the chain is the development of new friction materials to solve immediate problems, a process which, though continually eased by the flow of knowledge from the longer-term programmes, nevertheless derives a great deal from intuition and past experience. In parallel with this work on friction and wear, detailed researches are carried out on the properties of the more important raw materials. Asbestos is a particularly important material since, being a fibrous inorganic mineral, it is associated with three essential properties, namely, heat resistance, strength and flexibility. Studies on this mineral are being carried out by means of X-ray diffraction, electron microscopy and other techniques, and the structure of chrysotile and one of the amphiboles, namely, Bolivian crocidolite, has been elucidated. The investigations are run parallel with work on other properties of asbestos in order to obtain the clearest understanding of its many important characteristics. Investigations are also made of natural oils and resins by means of infra-red spectroscopy and liquid and gas chromatography, not only with the object of seeking a correlation between their properties and the friction and wear of the final product, but also to ensure that when complex mixes are involved, the components can be identified so that the activity of the oils can be maintained constant by a suitable system of blending. Mechanical investigations are used to examine the

behaviour of the many different types of brakes and to record the conditions to which they are subjected in service. Surface temperature is one of the many important variables, and both its measurement and calculation are of vital importance.

The Test House contains test dynamometers of the inertia type for examining experimental materials under conditions that can be encountered in service. The machines are, in the main, automatic in operation and are self-recording, and permit a much closer control and therefore a better opportunity for studying the various factors affecting behaviour. The larger machines are designed to fit the types of brakes used on aircraft, heavy transport, railways and industrial brakes, while on the smaller machines provision is made to fit brakes varying from automotive to bicycle. The workshop adjoining the Test House is fully equipped to make all but the large parts of the various test machines, and the laboratories situated on a second floor over the workshop and test car garage are concerned with the design and construction of the various servo and other devices needed for the operation and control of the machines. Towards the end of the wing is a test car garage which houses a fleet of test vehicles, including motorcycles, large and small saloon cars, high-performance sports cars, lorries and buses. The vehicles are all fitted with instruments of various types which, in effect, make them mobile laboratories.

Although the laboratories stand adjacent to the factory, it was considered essential for them to be self-contained, and to this end the pilot-plant laboratory is equipped with all the production plant and finishing apparatus required to make prototype materials. Such provision not only enables development staff to plan their work more easily, but also frees production plant in the factory from costly interruptions. One section of this wing is allocated to the Production Plant Development Section, which carries out all the proving trials that may be necessary on new plant of a novel design. This design section works not only in close co-operation with the pilot plant section, but also with those chemists and physicists who are concerned with ideas still only in their embryonic stage.

Even this brief description of the laboratories should serve to show that the study of friction and the manufacture of friction materials involves contributions from chemists, engineers, metallurgists, mathematicians, statisticians, physicists and physical chemists. Indeed, the added interest gained from co-operating with so many different disciplines serves to render a fascinating study exhilarating.

The architects for the new Ferodo Research Laboratories were Messrs. Ashworth and Fletcher; the quantity surveyors Messrs. Worrell and Kelly; and the consulting engineers Messrs. R. W. Gregory and Partners.

THE SCIENCE MASTERS' ASSOCIATION

ANNUAL MEETING

THE annual meeting of the Science Masters' Association was held in University College and Queen Elizabeth College, London, during December 30–January 2, and was attended by a thousand members and many guests; this number included members from Canada, India, Pakistan, South and West Africa, and the United States. Among them was Prof. R. W. Lefler, of Purdue University, Indiana, who is at present acting as adviser in physics to the Nationalist University in Formosa.

The meeting began with the presidential address by Sir Lawrence Bragg, who outlined the past history and present policy of the Royal Institution. The second evening was occupied by a lecture by Dr. H. Lister on the recent Trans-Antarctic Expedition; this was notable for the splendid coloured slides.

The programme of morning lectures by members of the staff of University College, together with guest lecturers from Harwell, the Brunel College of Technology, the London Institute of Education, and Woodberry Down School, London, has already been given in *Nature* (December 6, p. 1554). One of these, that by Mr. H. G. Conway, of Short Brothers and Harland, Belfast, on "New Engineering Problems in Aeronautics", was attended by Air Marshal Sir Owen Jones, president of the Institution of Mechanical Engineers, and other members of the Institution.

The Manufacturers' and Publishers' Exhibitions, held in University College, were larger than ever before, with the result that the exhibition of apparatus and other items brought by members had to be housed in Queen Elizabeth College. Among these exhibits was an intensely interesting historic one, namely, Faraday's bible, shown by Mr. E. H. Duckworth, of Cheltenham; this contained Faraday's autograph and his original marking of important texts.

The Science and Religion Group was addressed by the Rev. Aubrey Vine, general secretary of the Free Church Federal Council.

An innovation was the organization, at the suggestion of Mr. D. G. Chisman, education officer of the Royal Institute of Chemistry, of evening receptions by that Institute and by the Institutes of Physics and of Biology. These were much appreciated by members, as was shown by the fact that applications to attend far exceeded the available accommodation. The afternoons were occupied by visits to university departments and to factories in or near London; for other members there were exhibitions of scientific films.

At the business meeting of the Association Mr. D. G. James, vice-chancellor of the University of Southampton, was elected president for the ensuing year: Dr. H. F. Boulind, of the University of Cambridge Department of Education, was elected chairman, and Mr. H. Lockett, of Taunton's School, Southampton, became the new annual meeting secretary. It was announced that a summer meeting would be held, during July 28–31, in the Durham Colleges of the University of Durham. The next annual meeting will be in the University of Southampton, during December 29–January 1, 1960.

The second half of the business meeting was occupied with the discussion of a resolution, moved by Mr. D. N. Underwood and Mr. E. Chanter, which read: "that this meeting disapproves of the contin-

ued teaching of General Science as a substitute for the separate sciences". The chairman, Mr. Charles Holt, said that it was somewhat unusual for resolutions of this kind to be brought forward at the business meeting, but that the General Committee had decided that a useful purpose would be served if it were discussed. The Committee had always tried to avoid "coming down heavily on any particular side in science, because it took the view that members were so differently situated that they might find they had different needs at different stages of the work".

Mr. Underwood said the decision to move the resolution had been taken during the annual meeting last year at Leeds; concern on the subject had also been shown in articles in the *School Science Review* and in many letters and comments which the proposers had received in favour of the resolution. They were concerned chiefly with the effect of the teaching of general science on potential scientists, and not so much with that on the weaker grammar school and modern school pupil. It was a fallacy to think that physics, chemistry, and biology could be lumped together as general science; they were different disciplines and should be taught by experts. General science meant reduced time for science and provided little opportunity for practical work.

Mr. Chanter, in seconding the motion, said that in general science "one had to go quickly from one thing to another and the pupil never really got his teeth into anything". He did not believe that was real education at all.

The discussion was opened by a member who said that the sciences were humanities, and that the most human things about them were the barriers between them; the thing about chemistry which made it not physics was one of the most charming things.

Mr. J. A. Storer suggested an amendment, which was supported by Mr. D. Lewis (Bristol). This was later withdrawn after Mr. R. H. Dyball (City of London School) had proposed the altered wording "that this meeting disapproves of the continued teaching of general science on an inadequate time allowance". Mr. H. P. Ramage (Gresham's School, Holt) supported Mr. Dyball, and said that if physics, chemistry, and biology, with some geology and astronomy, were studied separately, it was still general science in the Association's meaning of the term. He would like to see enough time given to teach all these subjects up to Advanced (*A*) level; but the original resolution, if carried, would be misquoted and give a very wrong impression. After further discussion, the amendment proposed by Mr. Dyball was carried by an overwhelming majority.

The second discussion was on the Association's policy statement, "Science and Education", which has been widely distributed in educational circles. This was introduced by the chairman-elect, Dr. H. F. Boulind, who said that the main reason for the statement was "dissatisfaction with the present state of science teaching; the country is not producing the scientists, technicians, and technologists required". Secondly, it is science which is shaping the modern world, and some reasonable standard of scientific understanding is essential for everyone. The statement was produced by a sub-committee of two physicists, two chemists, and two biologists, and had been almost unanimously approved by the General Committee. It had been discussed with the Minister of Education and with Under-Secretaries, with the Central Advisory Committee, and with the Secondary School Examinations Committee; later work had been undertaken by specialist panels. Among the recommendations were the following: "that all pupils should follow an adequate course in science up to the end of the fifth-form year"; that "science should be studied by all pupils in the sixth form", and that "subject specialization should be retained in the sixth form but that *A* level syllabuses should be reduced in factual content".

In the discussion, a member said the problem should be considered from the point of view of the non-scientist; many important subjects are left out at Ordinary (*O*) level, so that pupils leaving at the age of 16 never touch them. Several members commented that the examining boards are favourable to a reduction in the factual content of examinations. Mr. W. H. Dovell (Lancing) thought that there are too many topics of interest only to the specialist in the *O* level course. He also asked for Dr. Boulind's views on the number of teaching periods a week. Dr. Boulind, in reply, said that nine periods a week should be adequate up to *O* level. Many members dissented at once. Mr. G. A. Lees (Collyer's School, Horsham) said that the policy statement was a very good effort, but that practical details as to time allowances were much more important. Further discussion centred on the question whether junior school science teaching should all be in the hands of one teacher or in those of specialists; on the influence which the Association might exert in introducing science into the common entrance examination for public schools; and on the effect of the statement on science teaching in modern schools and comprehensive schools. The chairman, in closing the discussion, mentioned that Dr. Boulind had been mainly responsible for the policy statement.

MANAGEMENT THEORY

THE third booklet in the "Problems of Progress in Industry" issued by the Department of Industrial and Scientific Research (Management and Technology. By Joan Woodward. Pp. 40. London: H.M. Stationery Office, 1958. 2*s*. 6*d*. net) describes a survey of the management organization of a hundred firms in south Essex. The survey attempted to discover whether the principles of organization laid down by an expanding body of management theory correlate with business success when put into practice. The survey was made between 1953 and 1957 by the Human Relations Research Unit of the South-East Essex Technical College, and after a basic survey of 91 per cent of manufacturing firms in south Essex with more than a hundred employees, twenty firms were studied more fully and detailed case-studies made of three firms in which production systems were mixed or changing. A full report of the research with a discussion of its theoretical implications is projected shortly under the title "Management

Organization: Theory and Practice". Among the large-batch and mass-production firms a relation was observed between conformity with the 'rules' of management and business success, and, within this limited range of production systems, observance of these 'rules' appears to increase administrative efficiency. Elsewhere, however, it appears that new 'rules' are needed and an alternative kind of organizational structure might be more appropriate. This does not invalidate management theory, which contains important and valuable information and ideas, but its limitations must be recognized and its principles subjected to critical analysis. Broadly, the survey brings out the way in which management organization varies according to the technical demands of the manufacturing process. The character of the basic functions of management, their chronological sequence, their integration and their relative importance to the success and survival of the business, all depend on the system of techniques in the particular firm, and they differ mainly between unit, mass and process production, all of which have different priorities.

Accordingly, a 'cause and effect' relation can be traced between a system of production and its organizational pattern, and, as a result, the probable organizational requirements predicted. The booklet emphasizes the danger in the tendency to teach the principles of administration as if they were scientific laws, when they are really little more than administrative expedients. Management studies can, so far, identify symptoms and remedies, but many more descriptive accounts, like that given in this booklet, of the circumstances in which different administrative expedients have proved successful are required to supplant traditional teaching. It is also concluded that technical changes not associated with changes in either objectives or the production system are unlikely to create much disturbance in the organizational pattern. Where, however, such changes are likely to create new 'situational demands', these could be foreseen by a systematic analysis of the new technology.

HUMAN RELATIONS IN INDUSTRY

IN its final report*, the Joint Committee on Human Relations in Industry, 1954–57, reviewing research projects approved since its first report, records that a practical inquiry into the effects of work study on human relations and organization at every level in the firm is well advanced, and further support was recommended for a team from the University of Manchester to continue to work on a large scale for three years on social factors affecting norms of production in British factories. An investigation into incentives for management had to be abandoned because suitable staff could not be found, but the National Institute of Industrial Psychology has been asked to undertake case-studies of the way in which individual firms have tackled the problem of foremanship.

Research in the field of industrial communication has been extended to cover the communication of technical information to firms from outside sources, and the inquiry conducted by the Oxford Institute of Statistics into factors affecting productivity has been extended to social factors in seeking for an explanation—psychological and social, as well as economic—of differences in productivity between and within the firms studied. Research into the community aspects of technological change in South Wales has centred around the exceptionally modern and highly mechanized Margam steel plant and, at Cardiff, on the impact made on the structure and procedure of the trades unions by the new steel plant.

The Acton Society Trust is making a case-study of the introduction of a management-development programme, and a study of educational provision and the needs of industry by the Tavistock Institute of Human Relations is also being supported. Work at the London School of Economics on the employment of women in industry was extended for two years, and some exploratory work was undertaken at the University of Edinburgh into the employment of older workers in industry.

Projects sponsored by the Joint Committee on Individual Efficiency in Industry fall into four groups. Those concerned with factors affecting equipment design and working conditions include two studies, by the Medical Research Council's Applied Psychology Research Unit at Cambridge, of manual controls of machine tools and of visual fatigue. The former greatly increased the speed of machine-working and reduced the number of errors and learning-time. Other studies were concerned with the design and layout of control points in steelworks, with special reference to crane-cabs, with machine controls in relation to equipment used in the boot and shoe industry and with the response to work in high temperatures in relation to age. Projects concerned with industrial engineering techniques embrace those of the nature and acquisition of industrial skills by the Department of Engineering Production at the University of Birmingham and of work-study rating, rest and fatigue allowances by the Department of Aircraft Economics at the College of Aeronautics, Cranfield.

Studies of problems of training include those by the National Institute of Industrial Psychology of methods of training in industrial skills; of conditions influencing the rate of learning of paced and unpaced motor tasks by the University of Oxford Institute of Experimental Psychology; of the methods used by the best operatives and their application to the training and re-training of operatives by the British Boot, Shoe and Allied Trades Research Association; and of reading efficiency by the Department of Commerce and Professional Studies at the North-Western Polytechnic, London.

Among the group of miscellaneous projects may be mentioned one on the effect of knowledge of results on production made by the Medical Research Council's Applied Psychology Research Unit; an investigation of indices commonly regarded as indicating the adjustment of the individual to his job; and a study of automation in relation to skill and the design of machines by the Department of Psychology, University of Cambridge.

* Department of Scientific and Industrial Research. Final Report of the Joint Committee on Human Relations in Industry 1954–57 and Report of the Joint Committee on Individual Efficiency in Industry 1953–57. Pp. vi+44. (London: H.M. Stationery Office, 1958.) 3s. net.

A CASE OF HUMAN INTERSEXUALITY HAVING A POSSIBLE *XXY* SEX-DETERMINING MECHANISM

By PATRICIA A. JACOBS and Dr. J. A. STRONG

Medical Research Council Group for Research on the General Effects of Radiation and Department for Endocrine and Metabolic Diseases, Western General Hospital and University of Edinburgh

RECENT improvements in techniques for the examination of human somatic chromosomes have made possible the study of the chromosome complement of human intersexes; consequently, it is now practicable to investigate the relationship in these cases between sex as determined by direct chromosome study, and sex as inferred from the study of 'nuclear sex chromatin' of the type described by Barr and Bertram[1]. This report is concerned with one of a series of patients with gonadal dysgenesis who are under investigation, and the particular feature of interest is the occurrence of 47 somatic chromosomes in contrast with the normal number of 46 in man.

In recent years the diploid chromosome number of 46 has been recorded in a large number of instances. In addition to the 60 cases cited in a previous publication[2] we have recorded a diploid number of 46 in bone marrow preparations from a further 40 European subjects. Kodani, on the other hand, has recently published results of counts made on testicular material from 36 Japanese and 8 American white males[3,4]. He claims that in 16 Japanese and 1 American there was a diploid number of 48; in 2 Japanese a diploid number of 47, and that in the remaining 13 Japanese and 7 whites the number was 46. He suggests that 46 is the basic diploid chromosome number for man, but in some instances there are additional "supernumerary chromosomes". The occurrence of this type of supernumerary chromosome, however, has not been reported previously among the vertebrates and awaits confirmation by other workers.

Our patient, an apparent male aged twenty-four, was presented as a case of gonadal dysgenesis with gynæcomastia and small testes associated with poor facial hair-growth and a high-pitched voice. Biopsy examination of testicular tissue showed the seminiferous tubules to be extremely hyalinized and atrophic, and also an apparent increase in the number of interstitial cells. Chromosome studies were attempted on part of this material, but no spermatogonial mitotic or meiotic divisions were seen. Smears made from both the buccal mucosa and the blood were examined by Dr. B. Lennox of the Department of Pathology, Western Infirmary, Glasgow, and found to demonstrate typical female morphology with regard to their nuclear sex chromatin.

Material obtained by sternal marrow puncture was used for investigating the somatic chromosomes. The technique used for culturing the material in the presence of colchicine and for making squash preparations has already been described[2].

The chromosomes were counted in 44 cells in metaphase and the results are shown in Table 1.

The majority of the cells contained 47 chromosomes, and in all those cells where the chromosomes were well fixed and spread, the count was undoubtedly 47 (Fig. 1). The apparent variation is in all probability due to technical errors. Fragments of cells containing chromosomes may become lost during the squashing process so that counts lower than the diploid number are obtained; and occasionally chromosomes split at the centromere and individual chromatids may be counted as chromosomes, giving a count higher than the diploid number[2].

Table 1

Chromosome No.	45	46	47	48	49
No. of cells	2	7	29	5	1

A study of the chromosome morphology in 8 cells of a suitably high standard showed that each of these had a normal male complement with the *Y* chromosome present, but that there was also an extra chromosome having a sub-median centromere occurring in the medium size range. In the normal male there are 15 chromosomes in this range, and in the female 16, all having sub-terminal or sub-median centromeres[2]. Owing to the slight variations in their size and morphology these chromosomes have proved difficult to pair, and it is in this category that the *X* chromosome is to be found.

There are strong grounds, both observational and genetic[5,6], for believing that human beings with chromatin-positive nuclei are genetic females having two *X* chromosomes. The fact that this patient is chromatin-positive and has an additional chromosome within the same size range as the *X*, as well as an apparently normal *Y*, makes it seem likely that he has the genetic constitution *XXY*. The possibility cannot be excluded, however, that the additional chromosome is an autosome carrying feminizing genes.

The presence of the extra chromosome might have been due to one or other of the parents having 47 chromosomes, and, therefore, chromosome studies were made on marrow specimens from both parents. Both were found to have a diploid number of 46 (Table 2), and analysis of cells of suitable quality showed the morphology of the chromosomes to be normal.

The occurrence of the extra chromosome therefore may be due to non-disjunction at either mitosis or meiosis during gametogenesis in one or other parent. Alternatively, it may be due to non-disjunction

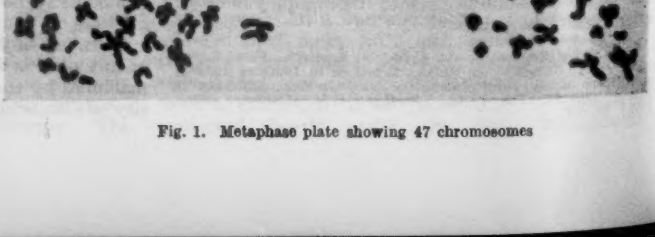

Fig. 1. Metaphase plate showing 47 chromosomes

Table 2

Father	Chromosome No.	44	45	46	47	48	49
32 cells counted	No. of cells	2	3	26	—	—	1
Mother	Chromosome No.	44	45	46	47	48	49
39 cells counted	No. of cells	1	3	33	2	—	—

occurring during the patient's very early embryological development, in which case there is a possibility that the patient may be a mosaic. Unfortunately, it is not possible with the techniques at present available to examine the chromosomes of tissues arising from different germ layers of the embryo.

We would like to thank Dr. B. Lennox of the Department of Pathology, Western Infirmary, Glasgow, for checking the nuclear sex of the preparations of buccal mucosa and blood and Miss M. Brunton for technical assistance.

A further report of this and other cases will follow.

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DISTRIBUTION OF NEWLY SYNTHESIZED DEOXYRIBONUCLEIC ACID IN DIVIDING CHROMOSOMES

By PHILIP S. WOODS and MARIE U. SCHAIRER
Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York

FROM autoradiographic studies with tritiated thymidine, newly synthesized deoxyribonucleic acid was shown to be equally distributed between both daughter chromosomes at anaphase by Taylor, Woods and Hughes[1] (Fig. 1*A* and *B*). When the labelled chromosomes were allowed to duplicate a second time in a medium containing no radioactive tracer, the isotope appeared in only one daughter chromosome of each sister pair (Fig. 1*C*). These findings differed markedly from the earlier conclusions of Plaut and Mazia[2], whose interpretations were based on the analysis of deoxyribonucleic acid labelled with carbon-14 in whole sets of daughter chromosomes in anaphase and telophase. Since they observed significant differences in the amount of label between the two daughter nuclei, they concluded that individual chromosomes were also labelled unequally. Recently, La Cour and Pelc[3] reported that unequal labelling could occur as a result of treatment with colchicine. Since in the experiment of Taylor *et al.* colchicine was present during the second synthesis and absent during the first, they suggested that colchicine may produce the unequal label.

In order to clarify the situation, the experiments of La Cour and Pelc were repeated. Since critical quantitative data were lacking in their experiments, information in the form of grain counts over a number of daughter chromosome pairs were collected and the chi-square test applied to determine any significant deviations between daughter halves.

The experiment went as follows: two seedlings of *Vicia faba* were grown in separate nutrient solutions containing tritiated thymidine (1·4 μgm./ml., 2·0 μc./ml.). One solution contained in addition 0·03 per cent colchicine. After 8 hr. the seedling which was treated with thymidine labelled with tritium alone was removed and placed in a non-labelled solution containing 0·03 per cent colchicine. After 6 additional hours two roots of this seedling were fixed, stained by the Feulgen squash procedure, and covered with autoradiographic stripping film. The other

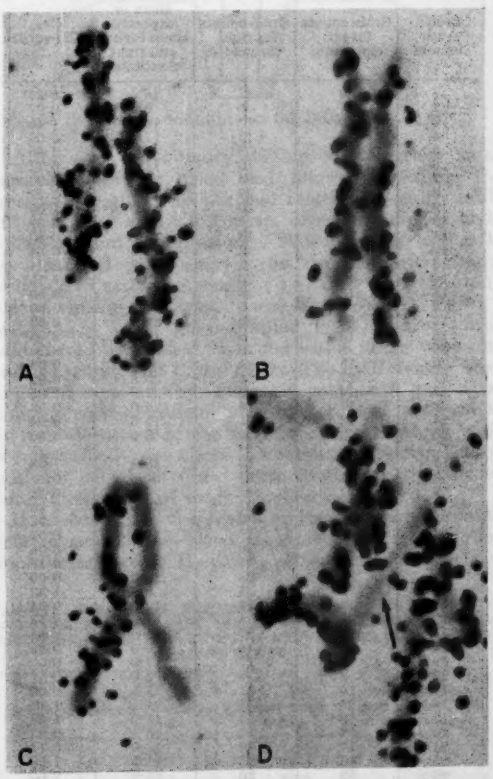

Fig. 1. *A*, autoradiograph of chromosome number 9 of second root of Table 2, colchicine present during synthesis; *B*, autoradiograph of chromosome number 4 of second root of Table 1, colchicine absent during synthesis; *C*, autoradiograph of chromosome from root treated with tritiated thymidine for 8 hr. followed by colchicine solution without tritiated thymidine for 30 hr.; *D*, autoradiograph of chromosomes from lightly squashed root from same seedling as that used in *B*

seedling was grown for a total of 12 hr. in the labelled thymidine–colchicine solution, and then two roots were fixed and processed identically with those of the first seedling. Definite criteria were followed for obtaining the grain counts. Since Fitzgerald *et al.*[4] have shown that 90 per cent of the beta-particles emitted by tritium are absorbed by filters (corrected to unit density) with a thickness of only $1{\cdot}2\mu$, labelled material would not be expected to register fully unless in direct contact with the photographic emulsion. Therefore, we have scored only those chromosomes that were seen by the phase-contrast microscope to be in direct contact with the emulsion. All other chromosomes such as those with varying thicknesses of cytoplasm or cell wall lying above were rejected. The first 25 chromosomes encountered where the criteria were fulfilled were counted for each of the four roots. Thus, 50 chromosomes were scored from each of two roots where colchicine was either present or absent during the period of synthesis of deoxyribonucleic acid.

Tables 1 and 2 show the results of these experiments. The fourth column of each table shows the mean or expected count for the left and right chromatid of each chromosome if equal labelling were to occur. The last column of each table shows the values of chi-square for each chromatid pair. It is clear from the high probability values that the observed grain count values do not differ significantly from the expected values of the 1 : 1 hypothesis. Apparently each daughter chromosome of a pair contains the same amount of new deoxyribonucleic acid. Also, there appears to be no difference between the two treatments. The presence or absence of colchicine during synthesis has no effect on the distribution of the labelled material. Fig. 1*A* and *B* illustrates two chromosomes that were counted for each treatment.

Table 1. Grain Counts of Fifty Chromosomes from Two *Vicia faba* Roots treated with Tritiated Thymidine for 8 hr. followed by non-labelled Colchicine Solution for 6 hr.

Chromosome number	Grain counts for left chromatid	Grain counts for right chromatid	Mean or expected count for left and right chromatid	Chi-square
1	20	17	18·5	0·24
2	7	6	6·5	0·07
3	20	13	16·5	1·48
4	18	17	17·5	0·03
5	33	38	35·5	0·35
6	9	13	11·0	0·72
7	17	23	20·0	0·90
8	17	27	22·0	2·27
9	6	10	8·0	1·00
10	7	8	7·5	0·07
11	18	13	15·5	0·80
12	13	12	12·5	0·04
13	11	12	11·5	0·04
14	48	30	39·0	4·15*
15	18	13	15·5	0·80
16	9	6	7·5	0·60
17	30	29	29·5	0·02
18	15	6	10·5	3·85*
19	8	10	9·0	0·22
20	15	14	14·5	0·03
21	27	26	26·5	0·02
22	14	20	17·0	1·05
23	17	13	15·0	0·53
24	12	11	11·5	0·04
25	3	9	6·0	3·00
1	12	19	15·5	1·58
2	21	21	21·0	0
3	7	11	9·0	0·88
4	21	27	24·0	0·75
5	4	9	6·5	1·92
6	12	16	14·0	0·57
7	11	10	10·5	0·05
8	11	13	12·0	0·17
9	21	23	22·0	0·09
10	8	4	6·0	1·33
11	12	13	12·5	0·04
12	10	4	7·0	2·57
13	16	27	21·5	2·81
14	22	16	19·0	0·95
15	17	17	17·0	0
16	8	8	8·0	0
17	16	21	18·5	0·68
18	26	17	21·5	1·88
19	8	14	11·0	1·63
20	16	15	15·5	0·03
21	17	19	18·0	0·11
22	10	15	12·5	1·00
23	30	20	25·0	2·00
24	4	5	4·5	0·11
25	4	3	3·5	0·14
			χ^2 (50 d.f.) = 43·61	
			P = 0·72	

* Significant at 5 per cent level

Table 2. Grain Counts of Fifty Chromosomes from Two *Vicia faba* Roots treated simultaneously with Tritiated Thymidine and Colchicine for 12 hr.

Chromosome number	Grain counts for left chromatid	Grain counts for right chromatid	Mean or expected count for left and right chromatid	Chi-square
1	5	6	5·5	0·09
2	8	9	8·5	0·06
3	3	3	3·0	0
4	5	4	4·5	0·11
5	13	8	10·5	1·19
6	9	5	7·0	1·14
7	7	6	6·5	0·08
8	7	5	6·0	0·33
9	8	7	7·5	0·07
10	7	10	8·5	0·53
11	32	30	31·0	0·06
12	12	7	9·5	1·32
13	9	20	14·5	4·17*
14	15	13	14·0	0·14
15	14	12	13·0	0·15
16	11	12	11·5	0·04
17	19	29	24·0	2·08
18	13	14	13·5	0·04
19	33	25	29·0	1·10
20	8	9	8·5	0·06
21	20	25	22·5	0·56
22	12	16	14·0	0·57
23	13	8	10·5	1·19
24	13	9	11·0	0·73
25	20	25	27·5	0·56
1	15	19	17·0	0·47
2	14	17	15·5	0·29
3	27	23	25·0	0·32
4	7	9	8·0	0·25
5	10	16	13·0	1·38
6	5	8	6·5	0·69
7	7	6	6·5	0·08
8	9	8	8·5	0·06
9	30	32	31·0	0·06
10	10	18	14·0	2·28
11	20	25	22·5	0·56
12	12	13	12·5	0·04
13	22	19	20·5	0·22
14	7	10	8·5	0·53
15	16	11	13·5	0·93
16	19	10	14·5	2·79
17	10	11	10·5	0·05
18	6	11	8·5	1·47
19	25	15	20·0	2·50
20	14	16	15·0	0·13
21	10	9	9·5	0·05
22	15	10	12·5	1·00
23	23	21	22·0	0·09
24	20	18	19·0	0·11
25	4	10	7·0	2·57
			χ^2 (50 d.f.) = 35·29	
			P = 0·94	

* Significant at 5 per cent level

Explanation of the findings of La Cour and Pelc[3] in the light of the present results should be considered. In our opinion, differential absorbance of tritium beta-particles by varying thicknesses of cytoplasm and other cellular components has not been sufficiently considered by these workers. It is probable that a chromosome labelled with tritium would not produce a satisfactory autoradiograph if it were separated from the emulsion during exposure by a layer of dry cytoplasm only a few microns thick.

It is clear that unless the chromosomes are heavily squashed so that the thickness of the absorbing layer is reduced to a minimum or is at least constant, inconsistent results would be expected. Fig. 1*D* shows a preparation in which one chromosome (arrow) of a group was farther from the emulsion than the others and in which the absorbing layer was thicker.

This investigation was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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SEPARATION AND ANALYSIS OF SCALE-RICH MATERIAL FROM WOOL

By Dr. J. H. BRADBURY

Wool Textile Research Laboratories, Commonwealth Scientific and Industrial Research Organization, Geelong, Australia

THE scales of animal fibres can be removed by physical methods such as abrasion[1–3], by enzymic methods[4], and by chemical methods[5–8]. The disadvantage of the chemical and enzymic methods is that it is impossible to estimate the degree of degradation of the scales which has occurred during the treatment. The abrasion technique has not been used for producing scales for analysis but rather to obtain descaled fibres for analysis[3] and dyeing experiments[2]. In this communication an abrasion technique is used to produce 0·5-mgm. amounts of scale-rich material which are quantitatively analysed for amino-acids by Levy's method[9].

The glass descaler is shown in Fig. 1. The central rod *A* comes to a chisel edge at *B*, which is fire-polished and has a cut made across it with a sharp glass-knife. This serves as an abrasive groove for the wool fibre *C*, which is held by tweezers and threaded through the holes *D* at either end of the descaler. When the fibre is rubbed once back and forth along the groove small particles are abraded and collect on the sides of the descaler. To ascertain the depth of abrasion of Lincoln 36's and Corriedale 46's fibres they were ring-dyed with 0·01 per cent Cibalan Bordeaux 3*BL* in 0·1 per cent glacial acetic acid at 80° C. for 1 hr., cross-sectioned with a Hardy microtome and the average depth of dyeing measured over 100 cross-sections. This amounted to 3·1 and 2·9 per cent of the diameter for Lincoln 36's and Corriedale 46's, respectively. Before use, each descaler was tested with the appropriate ring-dyed fibres and the scale material examined under the microscope. In every case it consisted of small, coloured pieces, showing that it originated from the outside layers of the fibre. A further check was made by calculating the average dimensions of the material abraded, from a knowledge of the percentage by weight removed from one side of the fibre, which is assumed to be circular in cross-section. When 0·50 per cent by weight of the fibre is abraded the calculated average depth of abrasion at the deepest point amounts to 2·0 per cent of the diameter. Since the thickness of the scales as determined by microscopy[10] is about 1·7 per cent of the diameter, the abraded material is very largely, but not entirely, scales.

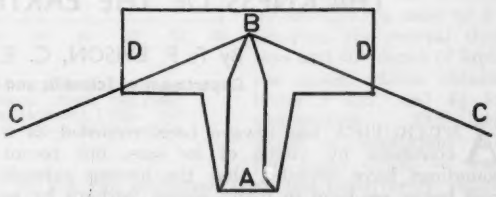

Fig. 1. Glass descaler, distance $DD \simeq 3$ cm., $AB \simeq 2$ cm.

After such preliminary testing each descaler was carefully cleaned, dried and allowed to come to constant weight at 65 per cent relative humidity and 20° C. After about 0·5 mgm. of scale material had collected from the abrasion of 200–400 fibres (cleaned as described previously[11]) the descaler was again weighed to constant weight ($\pm 5 \times 10^{-3}$ mgm.) and placed inside a large, carefully cleaned 'Pyrex' test-tube. Twice glass-distilled 6 *M* hydrochloric acid (2 ml.) was added, the tube sealed under vacuum and heated at 105° C. for 16 hr. The hydrolysate was dinitrophenylated by Sanger's method[12] and the dinitrophenyl-amino-acids separated and estimated by a modification of the method of Levy[9] which will be described elsewhere. Similar quadruplicate analyses of whole fibres were made for the three different samples of wool recorded in Table 1.

It is clear that the scales are rich in proline and poor in aspartic acid and glutamic acid in all three wools examined. However, substantial differences

Table 1. Percentage Deviations of Amino-acid Content of Scale-rich Material from that of Whole Fibre which are significant at the 1 per cent Probability Level unless otherwise stated

Amino-acid	Lincoln 36's (virgin wool, tips removed)	Lincoln 36's (obtained in top form)	Corriedale 46's (virgin wool, tips removed)
Asp	− 27·5*	− 14·2†	− 24·1
Glu		− 13·0	− 22·7*
Pro	+ 33·0	+ 20·6	+ 27·3*
Cys	—	+ 21·8	—
Gly	+ 38·7	—	—

* Significant at the 5 per cent probability level.
† $P = 0{\cdot}068$ in this case due to poor separation of DNP-Asp from DNP-Glu in some of the experiments.

between types may be expected since the scales are rich in glycine in Lincoln 36's virgin wool and rich in cystine in Lincoln 36's top. Scale material produced by various degradative chemical methods is reported to contain more cystine and less tyrosine than the whole fibre[5–8]. The differences between such results and those given here are due to the different methods of separating the scale material and possible variations in composition between various samples of wool and between the three histological components of the scales[13].

It is perhaps significant that the scales which are known to be amorphous[5,14,15] have a higher proline content than the fibrous cortex[16]. However, the large differences in chemical reactivity between cuticle and cortex are probably due more to differences at the molecular and higher levels of organization than to differences in chemical composition.

I thank Dr. M. Lipson for suggesting this line of work, Mr. J. Delmenico for advice on dyeing and microscopy, and various colleagues for helpful discussions. Mr. H. Groll has rendered valuable technical assistance.

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THICKNESS OF THE EARTH'S CRUST IN ANTARCTICA

By F. F. EVISON, C. E. INGHAM and R. H. ORR
Department of Scientific and Industrial Research, New Zealand

ANTARCTICA has always been regarded as a continent by virtue of its size, but recent soundings have revealed that the ice-cap extends well below sea-level in many places, perhaps by as much as 2,000 metres. Whether the surface of the rock in such areas would remain submerged if the ice were to melt is largely a question of the ensuing isostatic adjustment. A more direct means of verifying the continental status of Antarctica is to determine the thickness of its crust, for all the established continents have a crustal thickness of 30–40 km. or more, in contrast to thicknesses of 5–15 km. in oceanic regions. The continental type of crust may be readily distinguished from the oceanic type by a study of the dispersion of earthquake waves travelling in the crustal layers, provided that suitably located shocks and seismograph stations are available. Dispersive waves from an earthquake of September 9, 1957, with epicentre (Fig. 1) in the south-east Indian Ocean (47¾° S., 101° E.), were recorded at Scott Base and Hallett Station after traversing Antarctica for approximately 2,000 km., that is, about half the total path. An analysis of these waves has shown that the thickness of the crust in eastern Antarctica is about 35 km.; thus the existence of a true antarctic continent is confirmed.

The analysis was facilitated by a study of records of the same earthquake at Mirny, which indicated further that the thickness of the crust beneath the south-east Indian Ocean is about 9 km. The stations from which these results have been obtained were participating in the antarctic programme for the International Geophysical Year. Columbia University seismographs were used at Hallett Station (U.S.A.–N.Z.), Benioff seismographs at Scott Base (N.Z.) and Kirnos seismographs at Mirny (U.S.S.R.).

The wave-paths, shown in Fig. 1, were peculiarly favourable for the present study. The waves arrived well dispersed at Scott Base and Hallett Station, and the dispersion was influenced to a suitable extent by the segment of the path across Antarctica. Close similarity could be assumed between the oceanic structure affecting these paths and that for the path to Mirny. Although all the paths were rather shorter than would ordinarily suffice to prevent confusion among different types of wave, the directions of arrival were nearly ideal. Thus Rayleigh waves were

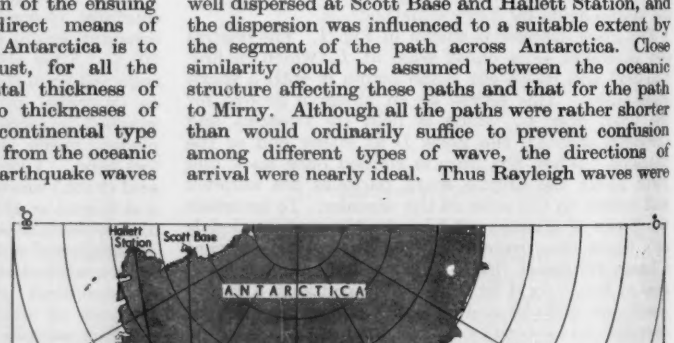

Fig. 1. Wave paths from earthquake epicentre to seismograph stations. (Polar stereographic projection)

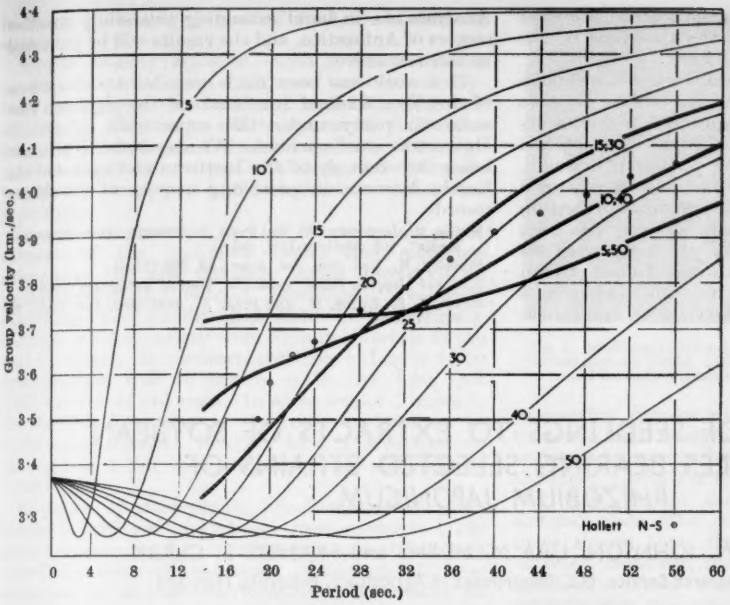

Fig. 2. Love wave dispersion. Theoretical curves are shown for crustal thicknesses from 5 km. to 50 km., with the following seismic constants. Shear velocities: crust, 3·47 km./sec.; mantle, 4·50 km./sec. Density ratio (mantle/crust), 1·283. The heavy curves are for composite paths, with one-half of the path at each of the thicknesses shown

clearly selected by instruments recording the east–west component at Scott Base and Hallett Station, and by the north–south instrument at Mirny, as well as by all the vertical instruments. Likewise, the Love wave was well recorded by the north–south instrument at Hallett Station; but the constants of the north–south instrument at Scott Base made it unsuitable for recording Love waves, and the path to Mirny was too short. The dispersion observations were analysed in the usual way[1], and interpreted by comparison with theoretical results. A three-layer model was adopted for the oceanic segment of the path, and a two-layer model for the land segment. The seismic velocities and densities of the crust and mantle respectively were assumed the same for Love and Rayleigh waves, and also for the oceanic and land segments.

The results for the Love wave recorded at Hallett Station are shown in Fig. 2. The family of theoretical curves was derived from parameters computed by Stoneley[2], each curve indicating the variation of group velocity with period for a particular crustal thickness. None of these curves approximates to the plotted observations, but a rather close fit is obtained by combining a pair of curves so as to allow for different thicknesses in the two segments of the path. With the segments equal in length, the composite group velocity for any period is the harmonic mean of the group velocities pertaining to each segment. Three such composite curves are shown in Fig. 2, from which it appears that the required crustal thicknesses are rather less than 40 km. for the antarctic segment and rather more than 10 km. for the oceanic segment.

The Rayleigh waves recorded at Scott Base, Hallett Station, and Mirny are analysed in Fig. 3 and provide an independent and rather more strongly supported solution. For an oceanic path the dispersion of Rayleigh waves differs from that of Love waves in being influenced by the liquid layer; thus two distinct families of curves are needed in considering a composite path. But the path to Mirny may be taken as purely oceanic, so in the present case a separate solution is obtained for the oceanic crust. A satisfactory fit to the Mirny observations was achieved by assuming a ratio of 2 : 1 between the crustal thickness and the depth of liquid, the actual values obtained being 9 km. and 4½ km. respectively. The latter value is consistent with the mean depth of water of 3·8 km. estimated from the bathymetric chart[3]; for studies of dispersion in the Pacific and Atlantic Oceans[4] have shown that the liquid layer usually includes unconsolidated bottom sediments to a

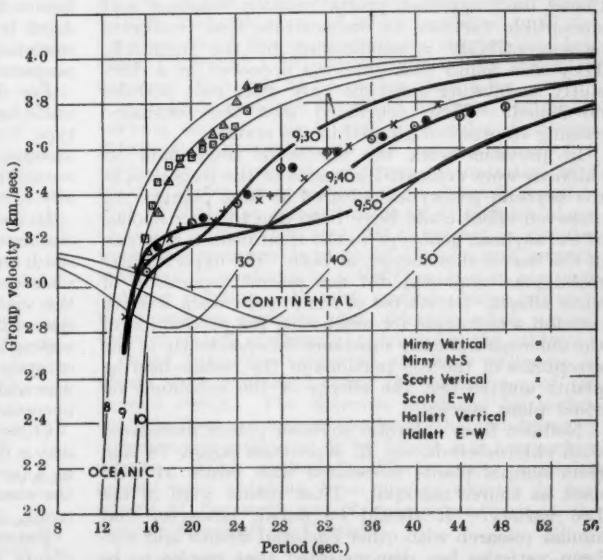

Fig. 3. Rayleigh wave dispersion. Theoretical oceanic curves are shown for crustal thicknesses of 8, 9 and 10 km., with depths of water equal to one-half the crustal thickness in each case. The continental curves are shown for crustal thicknesses of 30, 40 and 50 km. The following seismic constants were assumed, in addition to those of Fig. 2. Compressional velocities: water, 1·52 km./sec.; crust, 6·01 km./sec.; mantle, 7·79 km./sec. Density ratio (crust/water), 2·65. The heavy curves are for composite paths, with one-half of the path at each of the thicknesses shown

thickness of 0·5–1·0 km. From the composite curves in Fig. 3 it is concluded that the thickness of the crust of Antarctica is about 35 km.

The estimated values of crustal thickness, although derived from a single earthquake, receive support from the consistency that has appeared in the results for Love waves and Rayleigh waves. The method does not offer a high degree of precision, however, because of the simple models that must be assumed in order to keep the labour of computation within reasonable limits. A preliminary analysis has been made for a second earthquake, which occurred on August 4, 1957, in the south-west Indian Ocean (45° S., 35° E.) (see Fig. 1); this also indicates a thickness of about 35 km. for the crust of Antarctica. Analyses are in hand extending this study to other sectors of Antarctica, and the results will be published in full elsewhere.

This work has been made possible by the efforts of the New Zealand International Geophysical Year antarctic party under the supervision of Dr. T. Hatherton, chief scientist. We also desire to acknowledge the courtesy of the Institute of Physics of the Earth, Moscow, in providing a copy of the Mirny records.

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RESPONSES OF SEEDLINGS TO EXTRACTS OF SOYBEAN NODULES BEARING SELECTED STRAINS OF *RHIZOBIUM JAPONICUM*

By HERBERT W. JOHNSON, URA M. MEANS and FRANCIS E. CLARK
Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

ERDMAN *et al.*[1] reported that some varieties of soybeans (*Glycine max* (L.) Merrill) develop chlorosis when nodulated with selected strains of *Rhizobium japonicum*, whereas other varieties do not. Johnson *et al.*[2] obtained differential responses of soybean genotypes to various environmental conditions and succeeded in producing chlorosis in varieties previously considered resistant by varying nutrient and substrate conditions. Johnson and Clark[3] used approach grafts between resistant and susceptible varieties to demonstrate that resistance or susceptibility is conditioned by the rootstock. They also found that chlorosis appeared in a difficultly nodulating genotype only after root nodules developed. They concluded that the substance causing chlorosis originated in the nodule.

In previous work the effects of treatments on chlorosis were evaluated in terms of the responses of the soybean genotypes cultured as host plants. An observed effect could have been due to the response of the soybean genotype to the treatment, a response of the bacterial genotype, or both. The experimental techniques employed did not permit separation of these effects. Objectives of the present work were to develop a technique for evaluating the production of the chlorosis-inducing substance independently of the symptoms of the top portions of the nodule-bearing plants and to test the effects of the substance on other plant species.

Nodules from chlorotic soybean plants inoculated with chlorosis-inducing *R. japonicum* strain 76 and from normal plants inoculated with strain 31 were used as source material. Host plants were of the Lee variety. It should be noted, however, that similar research with other bacterial strains and soybean varieties has demonstrated that results to be reported are not peculiar to the source materials of this study. In preliminary work, weighed amounts of fresh nodules were macerated in a crucible in measured quantities of water and the suspension filtered. Such filtrates supported luxuriant growth of micro-organisms, and their chlorosis-inducing properties deteriorated rapidly. Filtrates autoclaved at 120° C. for 20 min. remained fully effective in inducing chlorosis, and once autoclaved they could be stored for several months at room temperature without measurable deterioration in activity. Sterile and initially highly active extracts deliberately contaminated with small quantities of soil lost their chlorosis-inducing activity during the first week after contamination. Extracts prepared from nodules frozen for as long as six months or from nodules dried in the greenhouse for as long as one month were not measurably different in activity from those prepared from fresh nodules.

For the tests reported here, nodule extract was made by blending fresh nodules in water in a Waring-type blendor in the proportion of 1 gm. of fresh nodules to 25 ml. of water. This stock extract was autoclaved at 120° C. for 20 min. and stored aseptically until used.

In testing for seedling responses, seeds were surface-sterilized or treated with 'Spergon', germinated in sand, and, at emergence, seedlings were transferred to vials containing the test solution. The capacity of the vials varied from 4 to 10 ml. depending on the size of the seedlings involved. These small reservoirs suffered depletion because of evaporation and consumptive intake of water. Additional test solution was added to them for replenishing the supply when necessary.

Chlorosis of the unifoliate and the first trifoliate leaves of soybean seedlings was scored from 1 (slight) to 4 (severe). Plants of other species were scored on the same scale and at comparable stages of development. Species tested are listed in Table 1.

Extracts from nodules of normal green soybean plants nodulated with strain 31 were included as checks in all experiments. None of the many seedlings grown in them developed chlorosis. To facilitate presentation of results, nodules or extracts from normal plants are referred to as the *N* type and those from chlorotic plants nodulated with strain 76 as the *C* type. Concentrations of the extracts are stated as

fractions, indicating 1 gm. of fresh nodule/ml. of water.

Soybean seedling responses. Both *N* and *C* extracts more concentrated than 1/50 were toxic to all soybean seedlings grown in them, and little chlorosis developed. The toxic effects appeared to be due primarily to damage of the root system. Development of branch roots was inhibited, and the main root quickly developed a greyish-brown water-soaked appearance.

Growth was retarded in 1/50 extracts, but slight chlorosis of the unifoliate leaves and moderate chlorosis of the first trifoliate leaves developed on seedlings grown in *C* extract. Seedlings of the highly susceptible Lee variety and those of the slightly susceptible *CNS* variety were equally chlorotic in the 1/50 *C* extract. In contrast, chlorosis of Lee in 1/100 concentration was as severe as in the 1/50, but *CNS* was free of chlorosis. In some lots of *C* extract, the Lee variety was as chlorotic in 1/200 as in 1/100 concentration; in other lots, it developed no chlorosis at 1/200, and only moderate chlorosis at 1/100.

Responses of other plants. Seedlings of 37 plant species in addition to soybeans were grown in 1/100 nodule extracts in the same manner as soybeans and scored for chlorotic response. Although all were evaluated on at least two occasions, the results do not necessarily indicate that the species which developed no chlorosis are resistant to the causative substance. All species were handled uniformly without effort to develop a technique particularly suited to a given species. It is quite possible that the technique employed was a limiting factor with some species but not with others.

Responses of the 38 species employed are summarized in Table 1. Of the seven species showing severe chlorosis, *Sorghum vulgare* showed onset of chlorosis two days after initial exposure, and the other six after three days. Average number of days for onset of chlorosis for the thirteen species listed as moderately chlorotic was 8·2, and for those listed as slightly chlorotic, 10·3 days. Seedlings of *Phaseolus vulgaris* and *P. lunatus* developed a mottled appearance after three weeks of exposure.

Chlorotic and normal seedlings of *Trifolium pratense* and *Sorghum vulgare* are shown in Fig. 1. Characteristics of seedling chlorosis for species other than soybeans were in general similar to those previously described for soybeans[2]. The chlorosis was restricted to new growth. Normal green leaves once fully formed did not develop chlorosis afterwards. In *S. vulgare* chlorosis developed first at the base of the second leaf and the entire leaf became chlorotic within a few days, but the first leaf remained green.

Damage to roots and general retardation of growth similar to those described for soybeans were observed

Table 1. Susceptibility of Seedlings of various Species to a Chlorosis Factor in selected Soybean Nodules

Severely chlorotic
Allium cepa, Digitaria sanguinalis, Sesamum indicum, Sorghum vulgare, S. vulgare (Sorgo), *Trifolium pratense, T. subterraneum.*
Moderately chlorotic
Cucumis sativus, Cucurbita pepo, Glycine max, Linum usitatissimum, Lotus corniculatus, L. uliginosus, Medicago sativa, Oryza sativa, Sorghum sudanense, Trifolium incarnatum, Vigna sinensis, Zea mays, Zinnia elegans.
Slightly chlorotic
Avena sativa, Lycopersicon esculentum, Vicia villosa.
Questionably chlorotic
Phaseolus lunatus, P. vulgaris.
Non-chlorotic
Amaranthus retroflexus, Arachis hypogaea, Brassica oleracea var. *acephala, B. rapa, Carthamus tinctorius, Festuca rubra, Gossypium hirsutum, Hordeum vulgare, Lolium multiflorum, Lupinus angustifolius, L. luteus, Raphanus sativus, Triticum aestivum.*

Fig. 1. Seedlings in 1/100 extracts of nodules from chlorotic and normal soybean plants. Left to right: *Sorghum vulgare*, chlorotic and normal; *Trifolium pratense*, chlorotic and normal

to varying degrees with other species. *Carthamus tinctorius* and *Gossypium hirsutum* appeared to be particularly susceptible, although neither developed chlorosis. Growth of soybeans in fresh *C* extract that had been filtered through a bacteriological-grade sintered glass filter was more rapid and chlorosis more severe than that of seedlings in unfiltered extract. However, this was not true for autoclaved extracts. Root damage caused by autoclaved extracts was not associated with various fractions separated by filtering or centrifuging.

The several experiments indicate that the water-soluble substance causing chlorosis is an entity differing from the substance causing toxicity.

S. vulgare seedlings exposed to 1/50 *C* extract for as little as 8 hr. before transfer to distilled water showed slight chlorosis by 48 hr. after the original exposure but recovered by the end of four days. Seedlings left in the extract for 24, 28, 32, 48, 50, 56, 72, 76 and 80 hr. developed progressively more intense chlorosis, some of which persisted until the termination of the experiment on the twelfth day. With 1/100 and 1/200 concentrations, the minimum exposure time was 24 hr. rather than 8 hr.

S. vulgare seedlings in mixed extracts containing varying amounts of *N* and *C* nodules developed chlorosis in proportion to the concentration of *C* nodules in the mixture. A given concentration of *C* nodules in the mixture resulted in chlorosis equal to that of the same concentration of *C* nodules alone. Chlorosis of seedlings in 1/100 *C* extract in distilled water was comparable in speed of onset and severity with that of seedlings in 1/100 *C* extract in complete nutrient solution. As with soybean plant cultures, there was no evidence that chlorosis when present was due to lack of essential nutrients in the substrate.

Other substances reported to cause chlorosis in plants were evaluated using the techniques of the present study. The chlorosis produced by nodule extracts does not appear similar to that produced by streptomycin sulphate[4] or by biuret and ammonium nitrate. With soybean nodule extracts, negative results were obtained from placing extract in leaf whorls of *Zea mays* and *S. vulgare* seedlings in the manner described by Curtis[5].

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LETTERS TO THE EDITORS

PHYSICAL SCIENCES

Phase Characteristics of Radio Signals received via the Ionosphere

A radio wave after reflexion from the ionosphere may be considered to consist of a specular component and a varying diffracted component[1], the latter being the result of random scattering from a large number of moving irregularities. If the effect at a point on the ground is considered, the diffracted component may be regarded as a cone of radiation having its apex at the point on the ground and its base spread over a large area of the ionosphere. The specular component appears as a coherent wave the arrival path of which is assumed to be along the axis of the diffraction cone and changes direction slowly compared with the fading rate of the signal[2]. Information concerning the amplitudes of the specular and diffracted components of a radio wave and of the angular spread of the cone of rays forming the diffracted component is an important factor in the design of some types of receiving aerials. This information may be obtained from the probability distribution of the phase-difference between the two signals which result from the reception of the radio wave at two aerials spaced apart by a known distance.

By spacing the aerials so far apart that the spatial phase-correlation in the diffracted component in the signals at the two aerials is negligible, the ratio of the power in the specular component to that in the diffracted component may be measured. Phase measurements provide a more sensitive method of detecting small specular components than amplitude measurements since the Rayleigh distribution of amplitude is not altered materially by the presence of a specular component unless its power is several times that of the diffracted component[1].

Using a result given by Rice[3] in his work on random noise, it may be shown that the combined probability distribution for the phase φ and amplitude R of the signal at one aerial is :

$$p(R, \varphi) = \frac{b^2 R}{\pi} \exp[-b^2 (R^2 + 1 - 2R \cos \varphi)]$$

where amplitudes are measured in terms of the specular component and where b^2 is the ratio of specular component power to diffracted component power. The phase-probability distribution may then be shown by integration to be :

$$p(\varphi) = \frac{1}{2\pi} \exp(-b^2) \times$$

$$[1 + b\pi^{1/2} \exp(b^2 \cos^2 \varphi) \cos \varphi \{1 + \mathrm{erf}(b \cos \varphi)\}]$$

An approximation to this distribution may be made by using a normal distribution with the skirts outside the range of variate $-\pi < \varphi \leqslant \pi$ folded over and added to the distribution within this range. The (unrestricted-variate) probability distribution for the phase-difference φ_d between two aerials is then a similar distribution having twice the variance of the unrestricted-variate distribution for one aerial if the correlation is zero.

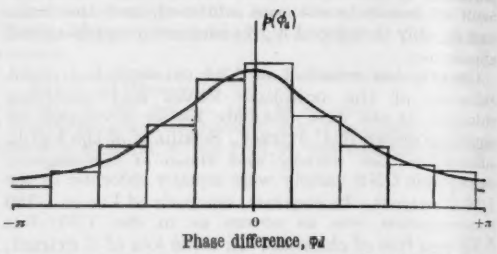

Fig. 1. Theoretical and measured phase probability distributions. Aerials widely spaced (26·04). WWV, 20·00 Mc./s. 1400 hr. February 10, 1958

With closer spacing between the aerials, measurements of the phase correlation between the signals enable the angular spread of the cone of rays forming the diffracted component to be estimated. The theory used in this case is similar to that used for direction-finding studies[4,5] with a correction applied to allow for the effects of the specular component.

Phase measurements of the type described have been made using a phase-distribution analyser. In this apparatus, the signals at the two aerials are changed in frequency to 1 kc./s. in three stages, common oscillators being used in order to preserve the phase difference between the signals. The 1-kc./s. signal derived from the first aerial is squared and differentiated to produce a pulse each time the waveform passes through zero in a positive direction. This pulse is arranged to open a gate which closes automatically after an interval of 0·1 msec. The closing of the first gate opens a second gate which also closes automatically after a similar interval, and this process is repeated through a chain of ten gates. The 1-kc./s. signal derived from the second aerial also causes a pulse to be generated each time the waveform passes through zero in a positive direction. This pulse is applied simultaneously to the ten gates associated with the first aerial and it will pass through one of the gates, depending on the phase difference between the signals at the two aerials. Ten counter units, each associated with one of the gates, are arranged to operate from the pulses passing through the gates so that after a suitable interval the numbers

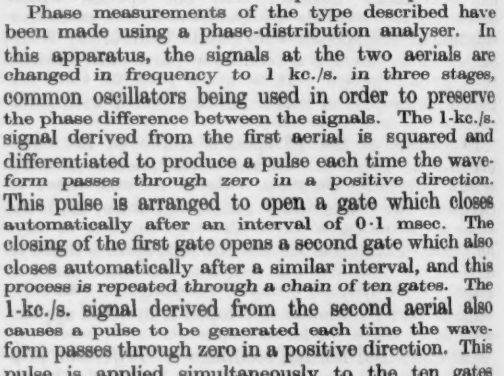

Fig. 2. Theoretical and measured phase probability distributions. Aerials close together (2·2λ)

displayed by the counters show the phase-difference distribution on a grouped-frequency basis.

The main advantage of this type of apparatus is the high rate of sampling which enables the probability distribution for several different aerial spacings to be determined before the effects of ionospheric tilting[2] produce significant changes in the statistical properties of the signal. Moreover, the distributions are displayed directly without the necessity for analysing chart records.

Typical 1-min. sample distributions are shown in Figs. 1 and 2 together with the theoretical distributions. The measurements were made on the standard frequency transmitter WWV (Washington, U.S.A.) operating on 20 Mc./s. at a time when a musa[6] receiver operating on 19·82 Mc./s. indicated that the transmission was virtually single-path. In Fig. 1, the peaking of the distribution was caused by the effects of a specular component alone. In Fig. 2, correlation in the diffracted component reduced the spread of the distribution still further. In the example quoted, the ratio of specular to diffracted power (b^2) was found to be 0·25, while the standard deviation of the angular spread of the diffraction cone in the vertical plane was approximately 6°. The vertical arrival angle derived from the means of the phase-difference distributions was 15°.

D. W. Morris
C. J. Hughes

Post Office Engineering Department,
Research Station, Dollis Hill,
London, N.W.2.

Dec. 5.

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Electric Strength and High-Field Conduction Current in *n*-Hexane

Several investigations of the electric strength and high-field conduction of *n*-hexane and similar hydrocarbon liquids have been made in recent years, and reproducible measurements have been reported, for example, by Lewis[1], Sharbaugh, Crowe and Cox[2], and by House[3]. New experiments of mine, however, indicate that nearly all these results were strongly influenced by the presence of dissolved oxygen.

Samples of dry filtered *n*-hexane were prepared using techniques described earlier[1,3] with the important addition that the liquid could be well degassed by evacuating the closed distillation system to about 10^{-2} mm. mercury with the liquid cooled to −90° C. The liquid could be maintained in the test cell in this degassed state or saturated with dry gas as required. The electric strength and conduction measurements were made with highly polished spherical electrodes (1 cm. diameter), 50μ apart, using either direct or 1·5 μsec. rectangular pulse voltages and adopting similar techniques to those given in refs. 1–3.

Typical results chosen from a large number of repeatable tests are given in Table 1 and demonstrate that when the air or oxygen content of the liquid is large the strength is high and the coefficient of variation small. In these conditions the results are in close agreement with those reported earlier[1,3].

Table 1. The Influence of Gas Content on the Electric Strength of *n*-Hexane. Values quoted are the means of not less than Ten Consecutive Measurements

Test voltage	Test liquid	Electrodes	Mean strength (MV./cm.)	Coefficient of variation (per cent)
d.c.	Degassed	Chromium	0·88	17·1
d.c.	High air content	Chromium	1·33	6·4
d.c.	High oxygen content	Chromium	1·33	6·2
d.c.	High nitrogen content	Chromium	0·89	10·7
1·5 μsec. pulse	High air content	Stainless steel	1·55	12·3
1·5 μsec. pulse	Degassed	Stainless steel	0·90	5·5

With degassed and nitrogen-saturated liquid the mean strengths are much lower, although the first few measurements with degassed liquid and fresh electrodes invariably gave high strengths. It was possible to change the liquid in the test cell from a degassed to an oxygenated state and vice versa several times with the electrodes unchanged and still maintain the strengths quoted in Table 1. It is important to note that the strength of a liquid with high air content showed no dependence upon pressure, in agreement with previous observations[1]; but for degassed liquid the strength decreased rapidly as the pressure was reduced below the atmospheric value.

The air content also influences the high-field conduction current. With degassed liquid the current fluctuated more and more violently as the field was raised and showed little tendency to become stabilized with time. On the other hand, a liquid with a high air content at atmospheric pressure was found to have a stable conduction current which could be measured at fields right up to 1·2 MV./cm. After the first voltage application reproducible current-voltage curves as in Fig. 1 could easily be obtained. It can be seen that the current is about a quarter of that reported by House[3] for the same electrode conditions. Even with hexane of commercial purity, curves almost identical to those given by House can be obtained provided the air content is large, without the necessity of a long conditioning process.

It is concluded that the oxygen content plays a vital part in determining the electric strength and conductivity of *n*-hexane. Experiments are proceeding to determine more precisely the role of oxygen

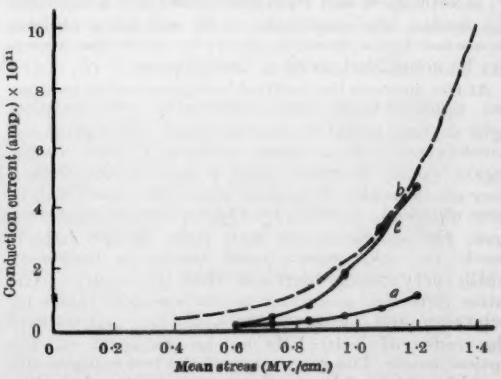

Fig. 1. Conduction current in *n*-hexane as a function of mean stress for a 50-μ gap and stainless steel electrodes. *a*, Spectroscopic grade hexane, air saturated at 760 mm. mercury; *b*, spectroscopic grade hexane, as reported by House (ref. 3); *c*, commercial grade hexane, saturated with air at 760 mm. mercury

and other gases in the breakdown processes, and the results will be published in due course. The present results are sufficient to show, however, that most of the high and reproducible values of the breakdown strength reported hitherto referred to *n*-hexane containing a substantial proportion of dissolved oxygen.

I wish to thank Prof. M. W. Humphrey Davies for the facilities made available to me. Thanks are also due to Dr. T. J. Lewis and other colleagues in the Department.

A. M. Sleiten

Electrical Engineering Department,
Queen Mary College,
Mile End Road,
London, E.1.
Dec. 15.

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A Proposed Method for the Measurement of the Velocity of Light

The precision of optical methods of measuring the velocity c of electromagnetic radiation in free space is restricted by the upper limit of the frequency at which a light wave may be amplitude-modulated. Bergstrand[1], in his recent measurements, used a modulation frequency of 10 Mc./sec. and consequently his base-lines were several km. long. The highest modulation frequency so far reported seems to be 300 Mc./sec. by Rao and Murty[2] using diffraction by ultrasonic waves in a crystal. It would be desirable to increase this limit so that an optical standard of length could be used directly in a determination of c. The correlation of photons in coherent light, which now seems well established[3], eliminates the necessity for a direct modulation of a light source. Two wave trains of optical frequencies ν_1 and ν_2, falling on a photosensitive surface, will give[4] a component in the photo-current at a frequency $(\nu_1 - \nu_2)$. If the wave trains are divided at a half-silvered mirror and fall on two photocells P_1 and P_2, the outputs S_1 and S_2 of these cells at a frequency $(\nu_1 - \nu_2)$ may be added and will interfere to give an output S_3, the amplitude of which depends on the relative phases of the two signals S_1 and S_2. It may readily be shown that if P_1 is kept fixed and P_2 is moved radially away from the source, the amplitude of S_3 will have minima separated by a distance $\frac{1}{2}c/(\nu_1 - \nu_2)$ if the source can be considered to be a distant point.

At the moment the method is impracticable because the signal-to-noise ratio achievable with existing light sources would be far too small. The proposed development[5] of a maser working in the visible region would, however, yield a light source with a very much smaller frequency width than has hitherto been obtained. Ideally, for the application suggested here, the source should emit light in two narrow bands $(\nu_1 - \nu_2)$ apart, each having a frequency width very much narrower than $(\nu_1 - \nu_2)$. The latter criterion, according to the example taken by Schawlow and Townes[5], could, if $(\nu_1 - \nu_2)$ were of the order of 1–10 kMc./s., be achieved in the optical maser. The separation of the two components could be obtained by the Zeeman splitting of the line emitted by the maser. The use of such a high value of $(\nu_1 - \nu_2)$ precludes the use of the conventional photomultiplier; but there seems no reason why a suitable photosensitive device[4] should not be designed for use at such frequencies.

The ultimate precision of this proposed method depends on the signal-to-noise ratio which can be achieved when: (*a*) the sizes of the light source and of the detecting surfaces are restricted within narrow limits, and (*b*) the frequency $(\nu_1 - \nu_2)$ is defined by a narrow-band receiver. Successful operation of the optical maser would allow the precise determination of c in the visible region over much shorter baselines, and possibly with higher precision than has hitherto been achieved. It would also provide a light source which would allow an even more convincing demonstration of photon correlation than that of Twiss, Little and Hanbury Brown[3].

J. H. Sanders

Clarendon Laboratory,
Oxford.
Dec. 13.

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An Experimental Method for Diffusion and Extraction Studies across the Liquid–Liquid Interface

The prototype apparatus shown schematically in Fig. 1 offers a promising experimental approach for diffusion and extraction studies across the interface between certain aqueous–organic systems uncomplicated by diffusion control in the adjacent bulk phases. It may also prove an effective probe of factors which influence the behaviour and 'structure' of the interface. The apparatus is operated in the following way. The substance being extracted (with tracer) is dissolved in 1 cm.^{3} of static aqueous phase placed so the aqueous surface formed at the bottom of the 'Teflon' slit (0·25 in. × 0·001 in.) is oriented lengthwise across the width of the rim, and about 0·006 in. above the wheel. Individual organic drops, at 1–5 drops/sec., are dropped on the 'Teflon' rim spinning at 10–200 r.p.m. Each drop is squeezed past the slit where it makes brief 'contact' with the aqueous phase before being almost entirely dragged away from the slit, wiped off, and collected at the bottom of the wheel. The activity/cm.^{3} of substance in the extract multiplied by the rate of production of drops is a measure of the 'apparent extraction rate', a parameter sensitive to the concentration of constituents involved in the extraction process.

In a study at 100 r.p.m. of the simultaneous extraction of radioactive iron(III) and cæsium(I) from a 1 *M* nitric acid/water solution with pure tributyl phosphate, the ratio of the activity of cæsium to that of iron in the extract was 0·80 ± 0·02 compared to the original value in water of 1·18 ± 0·03. The activity/cm.^{3} in this extract was about 10^{-3} times that of the aqueous phase, but it was about ten times more than is picked up by tributyl phosphate in a single equilibrium extraction with the same volume. These results indicate that a 'stable' interface with diffusive flow across it was achieved and that a differential rate of extraction in favour of iron(III) over cæsium(I) was obtained. Furthermore, the ratio of cæsium to iron in the extract differed markedly from the equilibrium value

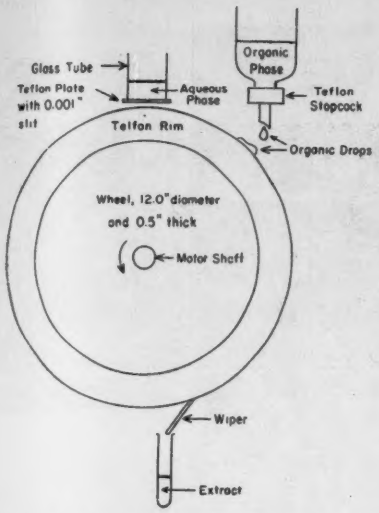

Fig. 1. Schematic cross-section of 'steady-state' interfacial extraction apparatus

of 2·0 for this phase, which is good evidence that the extraction depends on irreversible processes. Using tributyl phosphate/kerosene mixtures for the organic phase, the apparent extraction rate of iron(III) is dependent on the concentrations of iron(III), nitric acid and tributyl phosphate. For example, the rate is approximately fifty times greater using pure tributyl phosphate than using pure kerosene under similar conditions. These qualitative results indicate the feasibility of determining the form of the kinetic rate expression and testing the mechanism of extraction processes by this approach.

Thus it is possible to maintain an aqueous–organic interface within a 'Teflon' slit while mechanically pulsing it as frequently as every 0·2 sec. with a fresh drop of organic phase. Each drop apparently perturbs the interfacial region for a period which can be varied easily from 10^{-1} to 10^{-3} sec., during which mechanical agitation in the bulk phases adjacent to the interface must occur. These actions help to prevent diffusion in the bulk phases from limiting diffusion across the interface. It is believed that the net effect of frequent pulsing is to establish a relatively constant gradient of concentration, with a 'sink' on the organic side, so that diffusion across the interface is essentially unidirectional. The periodic pulsing of successive drops appears to be quite crucial to the success of this method, for the interface is unstable with a continuous flow of organic phase.

For processes between a two-phase liquid system, the method is somewhat analogous to the pulsing methods described by Eigen[1] for homogeneous systems. If it can be perfected, it may be possible to measure extraction rates and the form of the rate expressions for diffusion in both directions across the interface and thus obtain ratios of rate constants independent of uncertain quantities such as 'phase contact time' and the detailed geometry of the interface. Such rate constant ratios should be related to the distribution coefficients obtained in equilibrium extractions.

The simple geometry of the apparatus lends itself to certain modifications to make it an even more useful probe of interfacial phenomena. For example, the extract can be recirculated for slower diffusion processes like those through ion-exchange and biological membranes. It can also be adapted for extraction studies with a temperature gradient imposed across the interface. One last feature should be mentioned; because 'Teflon' is a non-conductor and can be charged, there exists the possibility of pulsing the interface with a variable electric field and of testing this effect on the extraction process. The instrument is now being modified for a more comprehensive investigation of its potentialities.

I am indebted to the Analytical Chemistry Division, ORNL, Oak Ridge, Tennessee, for the construction of the apparatus and the use of its facilities to obtain these experimental results while I was a summer participant supported by the Oak Ridge Institute of Nuclear Studies from June to September 1958. I also wish to thank B. A. Soldano, W. Baldwin and D. Harrison for valuable discussions.

THOMAS W. MARTIN

Department of Chemistry,
Vanderbilt University,
Box 1506,
Nashville 5, Tennessee.

Oct. 16.

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Measurement of the Flocculation of Suspensions

AT the Third International Rheological Congress held in Germany in September 1958, Prof. R. Fahraeus, in a paper entitled "The Influence of the Rouleau Formation of the Erythrocytes on the Rheology of the Blood", described a method which he had developed for measuring the degree of coagulation present in a blood sample. The procedure was to filter the blood through a stack of filter papers and to measure the quantity retained on each. The greater the coagulation of the blood, the less the number of papers through which it could penetrate.

I have recently tried this technique for measuring the degree of flocculation of a clay suspension. 100 ml. of a 2 per cent suspension (by weight) was poured into a pressure filter which contained a stack of papers 9 cm. in diameter and a pressure of approximately 15 lb./sq. in. was applied. At the top of Fig. 1 may

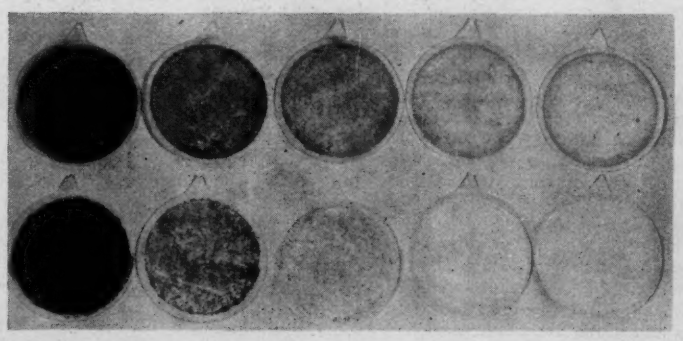

Fig. 1

be seen the successive papers obtained with a suspension (in distilled water) of a coal-measure shale from the Grove seam in the West Midlands. At the bottom are papers obtained after 5 ml. of a 1 per cent solution of potash alum had been added to 100 ml. of the same suspension in order to produce flocculation of the solid matter. The reduction in the penetration of the filter papers (Whatman No. 4) is quite evident and should be amenable to quantitative measurement.

Thus it appears that Prof. Fahraeus's technique could offer a method not only for measuring the coagulation of blood but also for the flocculation of shale and clay suspensions.

R. L. Whitmore

Department of Mining and Fuels,
University of Nottingham.
Dec. 19.

Etch Pits on Calcite Cleavage Faces

This communication describes some of the effects observed on calcite cleavage faces after etching with dilute acid. Puchegger[1] has reported etch pits which formed a rib-like pattern on either side of scratch marks made on calcite. More striking features are described here.

Rhombohedral crystals, freshly cleaved from a large block of natural calcite, were etched and examined on each face in turn. Etching was carried out in 1 per cent nitric acid, after which the crystals were washed with distilled water, ethanol and carbon tetrachloride, and dried. Etching times varied from 5 sec. to 5 min. The crystals were examined and photographed on a Vickers projection microscope using oblique illumination by a mercury-vapour lamp with yellow-green filter.

The same general features were found on each face and no features were found to be peculiar to any single face or pair of opposite faces. Numerous etch pits were observed occurring singly, in small groups, and in lines of overlapping pits. No pits were observed on unetched faces. Three types of pit were observed; three-sided 'flat iron' pits depicted in Fig. 1*a*, referred to hereinafter as type 1; four-sided pits (Fig. 1*b*), type 2; and five-sided pits (Fig. 1*c*), type 3. Types 2 and 3 predominated. Steps were observed on the faces of many of these pits and may be seen in Fig. 1*d*. Some pits retain their shape for long etching times, others cease to increase in depth relative to the general surface-level after a few seconds etching and then only increase in area to give flat-bottomed pits, some of which may be seen in Figs. 1*a* and *b*. These pits eventually overlap others and fade out to leave a shadowy outline. New pits sometimes start to grow in the flat bottom of an old one. Some pits persist for several minutes etching time and become visible to the naked eye; such pits have very rounded sides. A few highly pitted regions were observed. They appear within 5 sec. etching and persist with little change in configuration for 3 min. or more (Fig. 1*e*). These pits were all of type 3; but in the figure dark shadow makes it difficult to distinguish the intersection of two of the faces.

Visible fracture planes in the crystal, both parallel to and along the diagonals of cleavage faces, etched into deep grooves (Fig. 1*f*) with a rib structure similar

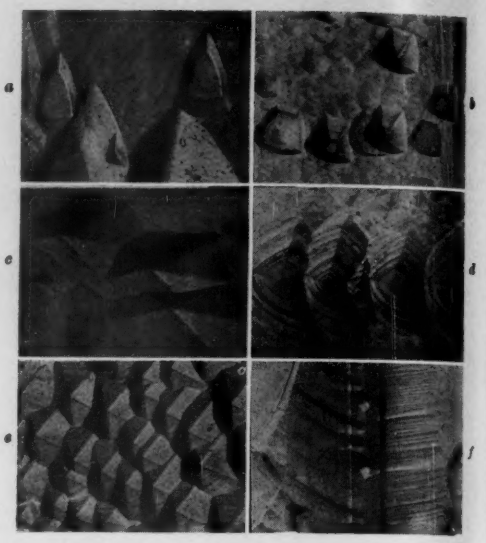

Fig. 1. *a*, Three-sided etch pits, type 1, 90 sec. etching; *b*, four-sided etch pits, type 2, 5 sec. etching; *c*, five-sided etch pits, type 3, 30 sec. etching; *d*, line of overlapping pits, 45 sec. etching; this line extended for more than 2 mm. on the crystal; *e*, highly pitted region, 60 sec. etching; *f*, etch groove at fracture plane—note width of ribbed region and pits in groove. (All *c*. × 100)

to but more pronounced than that observed by Puchegger[1]. These grooves sometimes contain a few deeper pits, as may be seen in Fig. 1*f*.

The fact that some pits lie in lines across the crystal (Fig. 1*d*) suggests that they are formed by preferential etching at the site of dislocations in the crystal[2]. The highly pitted regions would be regions of high disorder. The width of the ribbed region in Fig. 1*f* seems to indicate that slip along a fracture plane in the crystal disturbs a large region on either side of the plane.

The steps on a pit face make it possible to determine if that face is flat or curved, if it is presumed that all steps are parallel to the original surface before etching. All faces on pits of type 3 are flat, all pits of type 2 have at least one curved face, while all faces on pits of type 1 are curved. Pits of types 1 and 2 are probably degenerate forms of type 3 pits. Replacement of a pair of flat faces in type 3 by one curved face would give type 2; at the same time two other faces often become slightly curved. Type 1 pits would be formed from type 2 by continuing two faces to meet at a point.

It is difficult to envisage a mechanism whereby a five-sided etch pit can arise in a rhombohedral crystal. In view of the importance of etch pits to dislocation theory, I feel it is worth directing the attention of others to these effects at the present time.

I am indebted to Mr. W. T. Denholm for discussions.

H. Watts

Bonython Laboratory,
South Australian School of Mines and Industries,
Adelaide.
Dec. 12.

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A Micrographic Study of Wear of Graphite

THE sheet-structure of graphite is well known[1]: upon microscopic examination of crystalline fragments, individual sheets exhibit some properties of a tough membrane when not torn transversely. Individually they may be lifted or peeled to uncover a flat surface, which is highly reflecting, and in thin layers transparent.

These properties make graphite ideally suited for microscopic observations of its wearing characteristics during the sliding process.

The details of this process for graphite had not been apparently discovered. For example, it was not known whether wear accrues from the mechanical action of edges, the adhesion through edge surfaces or across massive facial areas, the tooling action of disoriented crystals, or by other unexpected effects.

Observations have been made here on natural graphite crystals oriented on the stage of a microscope under bright field epi-illumination. Initially the basal plane of the crystal was mounted rigidly and held flat and normal to the incident light beam. With selected adjustments of the illumination, steps, faults and surface impurities became prominent, and slight disturbances to a shallow depth within the crystal were found to produce faint shadows indicating the nature and extent of the stresses or damage.

During this work at magnifications up to 320, the graphite faces were explored by metal micro-probes or polished needles using the smooth conical section near the tip. The geometry of the arrangement made possible the estimation of the real contact area in relation to the area visibly disturbed by the contact.

This disturbed area was found to be very much greater than that bounded merely by the contact dimensions of the probe. Upon application of pressure of the probe against the graphite, a few sharp shadow lines frequently developed within the graphite surface adjacent to the contact spot. These spread to the sides or in advance of the probe and travelled with it as it traversed the basal plane surface. These shadow lines were usually straight, and extended to distances as much as a hundred times the contact radius. Some were completely elastic, disappearing upon withdrawal of the probe or oscillating back and forth if the probe was made to vibrate gently upon the surface.

By interference microscopy it was found that the shadow lines in representative cases could be the boundaries of basal plane area which had undergone minute localized tilting near the contact region, amounting to a few hundred angstroms of tilt across the entire field of view.

These optical line patterns on the graphite undergoing rubbing are interpreted tentatively as fault or dislocation regions at the intersectional boundaries between original planes and disturbed planes. This interpretation implies reversible atomic separations transversely through the basal planes in patterns sufficiently broad to be observed as narrow lines with an optical microscope. Disturbances of this kind should involve a contribution to the frictional work, and they indicate one of the characteristic features in the sliding process which may precede the onset of wear.

The appearance of these optical line patterns emanating beyond the region of real contact suggests that the shearing and ploughing terms, generally accepted as descriptive of the main processes involved in sliding solids, may not be sufficient to describe the wear and friction occurring on the surfaces of lamellar solids. A new description of the graphite-wearing process following from the detailed microscopic observations will appear in due course.

ROBERT H. SAVAGE
General Electric Research Laboratory,
Schenectady, New York.
Nov. 18.

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Low-temperature Specific Heats of Body-centred Cubic Chromium–Iron and Chromium–Manganese Alloys

THE electronic structure of the transition elements in the metallic state is not yet fully understood. It may be hoped that additional information with regard to the electronic specific heat coefficients and other physical properties of these elements, as well as of their alloys, will help in the interpretation of their structure.

On the basis of the band structure of the transition elements and of the usual interpretation of the electronic specific heat coefficient, it should be possible to trace the shape of the partially filled *d*-band, that is, the density of states as a function of the electron concentration, by determining experimentally the electronic specific heat coefficient γ for a series of solid solution alloys of a given crystal structure.

In the present work, low-temperature specific heats were determined for body-centred cubic binary solid solutions in the chromium–iron and chromium–manganese systems. Specific heats were measured in the temperature range of 1·6°–4·2° K. to an estimated accuracy of ± 2 per cent for most alloys. For a few alloys, where the specific heat depends very sensitively on the alloy composition, the accuracy may have been as low as ± 5 per cent. The alloy specimens were prepared by induction melting from electrolytically refined metals and homogenized by annealing for at least three days at 1,170° C. in a purified gas mixture of 92 per cent helium and 8 per cent hydrogen, and quenched to room temperature.

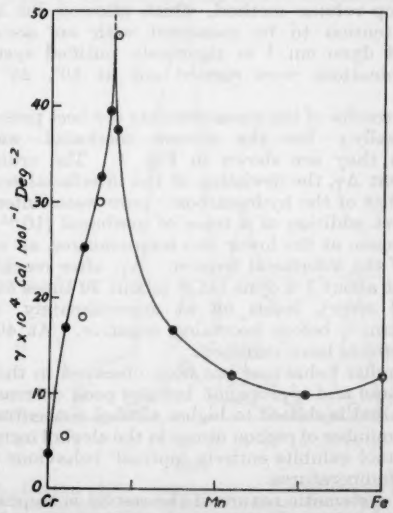

Fig. 1. Coefficient of temperature-linear term of low-temperature specific heat vs. electron concentration. ●, Chromium–iron alloys; ○, chromium–manganese alloys

The low-temperature specific heats were analysed by means of the usual C_v/T vs. T^2 extrapolation to $T = 0$, to obtain the coefficient γ of the temperature-linear term. These γ values are plotted in Fig. 1 as a function of the electron concentration. The γ for iron was measured and found to be in excellent agreement with the value reported by Keesom and Kurrelmeyer[1]. The γ for $Cr_{0.44}Fe_{0.56}$, as determined by Hoare and Matthews[2], is plotted in Fig. 1, and it fits very well the curve for chromium–iron alloys. A large peak occurs at $Cr_{0.81}Fe_{0.19}$, with a γ value more than ten times that of chromium[3]. The fact that similarly high γ values were found also for specimens in the chromium–manganese alloy series at corresponding electron concentrations suggests that the observed effect may be related to characteristic values of the electron concentration. However, it is as yet uncertain whether or not the γ-peak should be interpreted simply in terms of density of states. The work on the low-temperature specific heat and on other physical properties of these alloys is continuing.

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C. T. Wei
C. H. Cheng
P. A. Beck

College of Engineering,
University of Illinois,
Urbana.
Dec. 2.

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Effect of Low Concentrations of Alcohols on the Interfacial Tension between Water and Hydrocarbons

A study has been made of the effect of low concentrations of *n*-aliphatic alcohols on the interfacial tension of water against hexane and octane. For this purpose an apparatus was designed, based on the drop-volume method, which allowed the interfacial tension to be measured with an accuracy of 0·04 dyne cm.$^{-1}$ in rigorously purified systems. Determinations were carried out at 10°, 25° and 40° C.

The results of the measurements are best presented graphically; for the system methanol – water – hexane they are shown in Fig. 1. The ordinates represent $\Delta\gamma$, the deviation of the interfacial tension from that of the hydrocarbon – pure water interface. The first addition of a trace of methanol (10^{-11} mol. l.$^{-1}$) causes, at the lower two temperatures, an elevation of the interfacial tension. $\Delta\gamma$, after reaching a peak at about 1·2 dyne cm.$^{-1}$ (about 30 times experimental error), levels off at approximately + 0·4 dyne cm.$^{-1}$, before becoming negative. At 40° C., these effects have vanished.

A similar behaviour has been observed in the case of ethanol and *n*-propanol, but the peak decreases in height and is shifted to higher alcohol concentrations as the number of carbon atoms in the alcohol increases. *n*-Butanol exhibits entirely 'normal' behaviour at all three temperatures.

The systematic nature of the results is emphasized by tabulating: (1) the maximum elevation of interfacial tension, and (2) the threshold concentrations

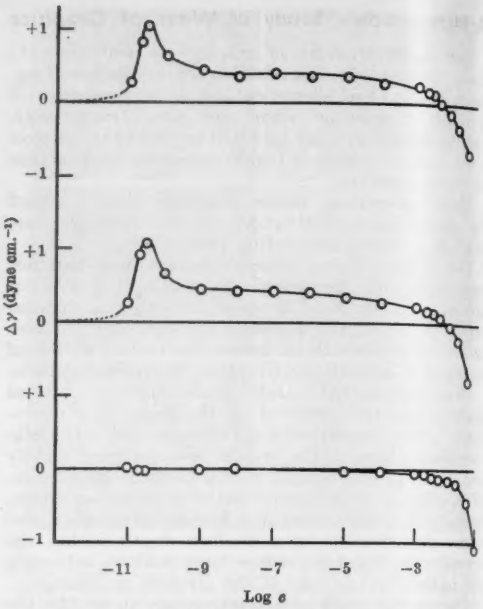

Fig. 1. Interfacial tension/concentration isotherms. Top, 10° C.; middle, 25° C.; bottom, 40° C.

above which normal, preferential adsorption of the solute occurs. This is done in Table 1.

When the thermodynamic functions associated with the generation of 1 cm.^{2} of interface, relative to those for the alcohol-free system, are calculated, it is found that the disappearance of the abnormalities between 25° and 40° C. is accompanied by an exaltation in ΔH, and an even more striking exaltation in $T\Delta S$. In order to ascertain whether the abnormalities disappear over a range of temperatures or at some definite temperature, interfacial tensions of several systems have been measured at temperatures between 25° and 40°. Similar measurements have also been made on the system hexane – pure water. In every case it is found that the abnormal behaviour disappears very suddenly between 33° and 37°. Even for the system hexane – water itself the slope of the interfacial tension/temperature curve exhibits a maximum in the same temperature-range. Fig. 2 shows the effect of temperature on the entropy (or relative entropy) of interface formation $\left(-\Delta S = \frac{d\gamma}{dT}\right)$. It is of interest to note that a similar type of curve is obtained for the entropy of surface formation of water, derived from the surface tension of water.

To show that the observed effects are not due to some peculiarity of the hydrocarbon normally used, determinations were carried out using *n*-octane as the oil phase. Identical results were obtained with this hydrocarbon. The abnormal effects are in all cases

Table 1

Alcohol	(1) Maximum $\Delta\gamma$ (dyne cm.$^{-1}$) 10°	25°	(2) Threshold concentration (mol. l.$^{-1}$) 10°	25°
MeOH	1·08	1·08	$6·7 \times 10^{-3}$	$8·5 \times 10^{-4}$
EtOH	0·70	0·72	$3·0 \times 10^{-3}$	$4·0 \times 10^{-4}$
PrOH	0·49	0·43	$0·55 \times 10^{-3}$	$0·3 \times 10^{-3}$

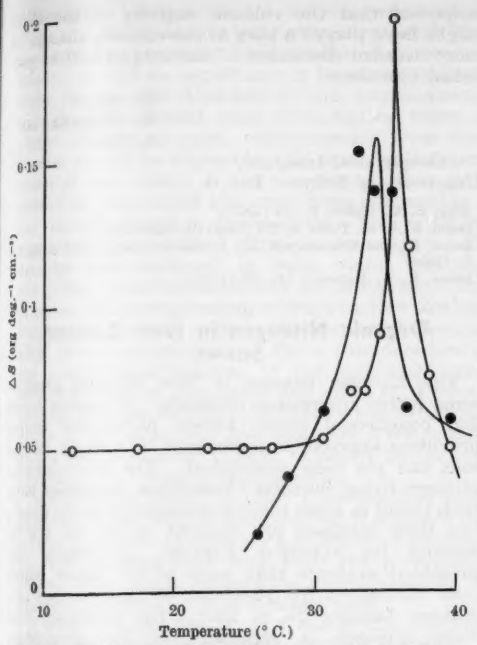

Fig. 2. Entropy of interface formation. ○, 6×10^{-4} *M* n-propanol/hexane (relative to water/hexane); ●, water hexane

abolished by the presence of hydrochloric acid in the aqueous phase.

A full account of the experiments carried out and a discussion of the abnormal effects obtained in terms of recent views on the structure of water and on the hydration of molecules will be published elsewhere.

F. Franks

Department of Chemistry,
Huddersfield College of Technology.

D. J. G. Ives

Department of Chemistry,
Birkbeck College,
London, W.C.1.
Nov. 27.

METEOROLOGY

Volcanoes as a Source of Atmospheric Ice Nuclei

In order to determine the nature and the concentration of ice-forming nuclei in the atmosphere, which are considered to play an important part in the initiation of rain, we have been carrying out, in Tokyo since April, measurements of the concentration of ice nuclei by means of an ice nuclei counter similar to that of Bigg[1]. The concentration is measured at temperatures of − 13° C., − 15° C. and − 20° C. It was found[2] that the concentration of ice nuclei was low in maritime air masses and high in the air masses which reached Japan from arid or semi-arid regions of the continent of Asia. Very high concentration in spring was found to be associated with the arrival of dust consisting of loess particles originating from the ground of the arid region of north China. The result of our experiment on loess particles which fell in Japan shows that they are effective as ice nuclei at temperatures below − 13° C. Despite the general tendency already mentioned, the concentration of ice nuclei in the atmosphere increased occasionally even in maritime air masses.

This phenomenon has been found to be related with eruptions of active volcanoes in Japan. In June and July 1957, Volcanoes Mihara, Aso, Suwanose and others were very active and erupted intermittently, ejecting ash high up into the atmosphere. The very high concentration observed during the period June 25–July 5 is considered to be due mainly to the ash from Volcano Mihara, which is at a distance of 100 km. to the south of Tokyo. The event which showed the most distinct and direct effect of volcanic ash was the eruption of Volcano Asama at 20h. 50m. on November 10, 1958. The eruption was the most violent among those in the past twenty years. After this eruption, Mt. Asama emitted coloured smoke (instead of white smoke as usually) continuously and erupted intermittently. The concentration of ice nuclei in the air observed in Tokyo is shown in Fig. 1, in which marked increase (40 times more than usual) in the nuclei concentration after the eruption is seen. The increase of concentration was large, but smaller than might have been expected from the scale of the eruption. It is considered to be due to a south-west wind, which carried most of the ash towards the north-east, while the measurement was made in Tokyo, which is situated to the south-east of the volcano. On November 12, the wind veered, smoke being observed to flow eastward and the concentration of nuclei increased. Minor peaks in the concentration seen in Fig. 1 were associated with the smaller eruptions which followed the violent one. The results of our laboratory experiments on volcanic materials from active volcanoes in Japan show that their property of serving as ice nuclei differs according to the nature of the material (Fig. 2) and that ash from Volcano Asama is very effective as previously described[3,4].

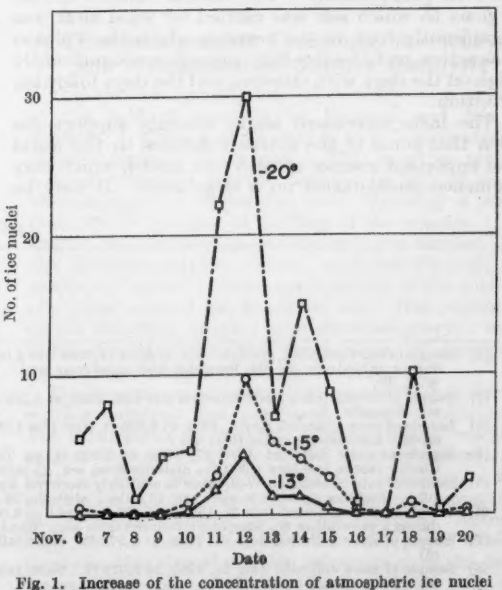

Fig. 1. Increase of the concentration of atmospheric ice nuclei due to eruptions of Volcano Asama. Measurement was made in Tokyo, 140 km. from the volcano. The greatest peak of the concentration corresponds to the most active period of eruptive activity

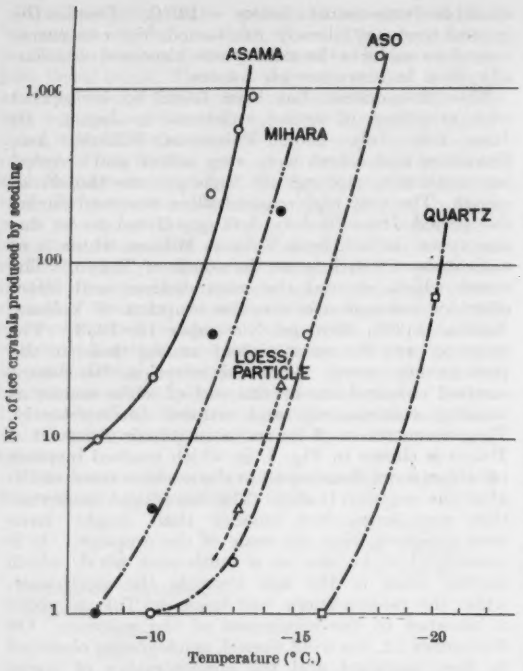

Fig. 2. Number of ice-crystals formed when 10 mgm. of each material is seeded into a cold box kept at the temperature indicated

Based on the statistical study of the relation between the amount of rainfall and the eruptions of Volcano Asama, we have suggested[3] that volcanic ash might be one of the important categories of ice nuclei in the atmosphere and play an important part in precipitation. The annual rainfall in the regions to which ash was carried by wind aloft was significantly high in the years in which the Volcano was active, and also the daily amount was significantly high on the days with eruption and the days following eruption.

The facts mentioned above strongly support the idea that some of the active volcanoes in the world are important sources of active ice nuclei, which may influence precipitation on a large scale. It may be suspected that the volcanic activity of the Earth might have played a part in the climatic change. A more detailed discussion of the subject will be published elsewhere.

K. Isono
M. Komabayasi
A. Ono

Geophysical Institute,
University of Tokyo. Dec. 1.

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Organic Nitrogen in New Zealand Snows

The nitrogen balance in New Zealand presents some rather interesting problems. In native forest and commercial exotic forests no natural source providing appreciable increments of nitrogen to the soils has yet been established. The non-symbiotic nitrogen-fixing bacteria *Clostridium butyricum* have been found in some tussock grassland soils by Ross[1]; but their numbers are thought to be too low to account for extensive fixation. Stevenson[2] has presented evidence that some of the higher plants may themselves fix quantities of nitrogen [see also *Nature*, January 24, p. 223]. The possibility that some nitrogen is supplied via the atmosphere has been investigated by me. Snow was collected above the vegetation line to avoid possibility of contamination by plant or animal debris. This was done in preference to the more conventional rain-water sampling, which is open to large contamination errors. Since the samples were collected in the early and mid-winter there is little possibility of contamination with pollen. The analyses of snow collected from various parts of New Zealand are shown in Table 1. The samples of snow were either kept frozen until analysis, or treated on collection with toluene to arrest any bacterial activity.

As can be seen from Table 1, New Zealand snow may contain up to 0·23 p.p.m. of bound nitrogen. No nitrogen is found as nitrate or nitrite, which are the classical nitrogen-cycle contributions from the atmosphere (products of electrical discharges). Perhaps the most interesting point in the above results is that sometimes most of the nitrogen is found in the 'albuminoid'[3] or organic nitrogen fraction.

Table 1

	Nitrogen content of snow samples (ref. 3)				
	NO_3^- (p.p.m.)	NO_2^- (p.p.m.)	NH_4^+ (p.p.m.)	Organic (albuminoid) (p.p.m.)	Total (p.p.m.)
(1) Sample of snow collected April 26, 1958, at 6,000 ft. (tree line 4,000 ft.) during snowstorm on Mt. Ruapehu (100 miles from sea). Wind W.-S.-W.	0	trace < 0·002	0·08	0·15	0·23
(2) Sample of freshly fallen snow collected April 26, 1958, at 5,300 ft. on Mt. Ruapehu	0	trace < 0·002	0·03	0·2	0·23
(3) Sample of snow collected May 3, 1958, at 6,000 ft. (tree line 4,000 ft.) on Mt. Egmont (20 miles from sea)	0	0	0·03	0·1	0·13
(4) Sample of snow collected April 27, 1958, at 7,000 ft. on Tasman Glacier (above tree line 4,000 ft., distance from sea, 25 miles)			0·1	0·12	0·22
(5) Sample of rain collected in Wellington in southerly storm on April 26, 1958. Distance up-wind to open sea, 10 miles; altitude, 10 ft.	0	0·002	0·27	0·2	0·47
(6) Sample of snow collected July 20, 1958, at 5,750 ft. (tree line 4,000 ft.) during a snowfall on Mt. Ruapehu (100 miles from sea). Wind, N.-W.	0	0	0·2	0·04	0·24
(7) Sample of snow collected July 21, 1958, at 6,575 ft. Same fall as in (6)	0	0	0·33	0·07	0·40
(8) Sample of snow collected July 21, 1958, at 8,070 ft. Same fall as in (6)	0	0	0·2	0·02	0·22
(9) Sample of snow collected August 2, 1958, at 6,000 ft. during snowstorm on Mt. Ruapehu	0	0·001	0·02	0·09	0·11

Albuminoid nitrogen is not usually measured by those who study atmospheric chemistry[4], and when measured has generally been attributed to contamination, though its significance is becoming realized[5]. Since the samples described in this communication were freshly collected snow from regions where no plants or animals exist, contamination from these sources should be negligible. No insoluble inorganic material was visible in the samples, so that cyclic terrestrial dust could not contribute appreciably to the nitrogen found in the samples. This is further supported by the lack of nitrate in the samples. The samples were collected in early winter and thus the pollen contribution would be negligible. Thus it seems that precipitation, at least in New Zealand, does in fact contain organic nitrogen which does not arise from contamination. From considerations of the geographical position of the sampling points with their lack of population and industry, I believe that the nitrogen found in the samples has its ultimate origin in the ocean and does represent a net transfer of nitrogen from ocean to land. This will be discussed in detail in a future communication.

If the results shown in Table 1 are taken as representative of the annual precipitation, it suggests that several pounds of nitrogen per acre are being deposited on New Zealand each year from the surrounding ocean. This might represent a contribution to the nitrogen economy of New Zealand soils.

I wish to thank Mr. H. J. Wood of the Dominion Laboratory, Wellington, for the analyses and Mr. A. J. Heine and Mr. G. Pallo, Department of Scientific and Industrial Research, for collecting samples.

A. T. Wilson

Division of Nuclear Sciences,
Private Bag, Lower Hutt,
New Zealand.

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CHEMISTRY

An Autoradiographic Method for distinguishing Samples labelled with Phosphorus-32 and Sulphur-35

Gillies[1] has recently described a method for examining insects labelled with sulphur-35 or phosphorus-32. The specimens were covered with two pieces of X-ray film, and autoradiographs made in the usual way. The low-energy sulphur β-particles (0·167 MeV.) were found to blacken only one film, while the high-energy phosphorus β-particles (1·71 MeV.) penetrated the first film and blackened the second also.

This useful method can be simplified by using film coated with photographic emulsion on both sides, as are most commercial X-ray films. In this laboratory, Kodak 'Kodirex' film is used for autoradiography of paper chromatograms, and with this material the two isotopes can be easily distinguished. If there is sufficient blackening of the film (such as is shown in the figure in Gillies's paper), a matt appearance is observed in reflected light and it can readily be seen whether one or both sides are affected. This can also be seen, even after much smaller exposures, by removing a little of the emulsion from both sides with a blunt scraper, either on the wet film after processing or after softening the gelatin on a dry film by moistening with 2 *N* sodium hydroxide. Examination of the film during photographic development is also helpful. Blackening of one side will not show on the other side of an uncleared film.

Gross over-exposure of a highly active sample should be avoided, since under these conditions even sulphur-35 will produce visible blackening on the second side of the film.

As would be expected, the effect of γ-rays is much smaller than that of β-particles on such a thin, light material as X-ray film. Thus bromine-82, with a β-particle energy of 0·44 MeV., produces a comparatively small amount of blackening on the second side of the film, although it is also a hard γ-emitter; more is produced by iodine-131, which has an average γ-energy less than that of bromine-82, but a higher β-energy (0·61 MeV. for 87 per cent of particles).

From the β-energies, using the equation given by Whitehouse and Putman[2], the half-thickness for various isotopes has been calculated, and thence the fraction of β-particles that would pass through 'Kodirex' film (which has a surface density of about 28 mgm./cm.²). Approximate values of this factor for a number of useful isotopes are: carbon-14 and sulphur-35, virtually nil; bromine-82, 0·23; iodine-131, 0·40; phosphorus-32, 0·82. These figures are consistent with the relative blackening of the two sides of the film judged subjectively, and suggest that it might be feasible to distinguish more than two isotopes by this method.

W. G. Duncombe

The Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent.

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A Chromatographic Technique for studying the Mechanism of Surface Catalysis

Gas chromatography has successfully been applied in the field of catalysis by Emmett and his co-workers[1–3], where it is referred to as a "microcatalytic chromatographic technique". This involves placing a small catalytic reactor directly on top of a gas chromatographic apparatus and injecting a small quantity of reactant at the top of the reactor. The slug of the reactant passes through the reactor, into the chromatographic column, and out through the analytical system; thus a rapid survey of the activity of a given catalyst can be carried out. This communication describes another new chromatographic technique for studying the mechanism of catalysis, measuring adsorption during reaction.

The importance of adsorption measurements during surface catalysis has previously been emphasized[4]. The adsorption on catalyst surface during reaction cannot be estimated from the separate adsorption data of each gas involved in the catalysis. The adsorption during reaction also depends upon the rate-controlling step of the overall reaction. Accordingly, if we could estimate the adsorption during catalysis, this should provide information on the rate-determining step of the reaction.

The adsorption measurements during surface catalysis can be carried out using the principle of gas

chromatography in the following way. The gaseous reactant passes through the chromatographic column charged with catalyst as a carrier gas such that not all the reactant would be consumed while it passes through the column. The sample gas is injected at the top of the catalyst column. The slug of the sample gas then passes through the catalyst on which the reaction occurs, and out through the analytical system, being carried by the reactant. The more strongly the sample gas is adsorbed the longer becomes the time of its retention. Accordingly, the adsorbability of the sample gas during the reaction can be estimated.

The separate adsorption of each gas on the catalyst can be estimated independently in a similar way using inert carrier gas instead of reactant, and from the dependence on temperature of the apparent retention time its heat of adsorption can be determined.

Palladium was deposited on 'Celite 545' according to the method described by Sano[5] in the ratio 1 : 20. The catalyst was charged in the adsorption column 12 m. long. The apparent initial retention times with various carrier gases and samples at 99° C. are listed in Table 1, where the rate of flow of the carrier gases was 15 c.c./min. and the zero apparent initial retention time corresponded to 22·0 min. It was recognized with a detector tube method[6] that the formic acid partly remained unreacted while it passed through the catalyst column at 99° C. and a sharp peak of the acid slug was observed when it was injected as a sample.

Table 1

Carrier gases	Samples	Apparent initial retention time (min.)
Nitrogen	Argon	0
Nitrogen	Carbon dioxide	0
Nitrogen	Hydrogen	8·5
Hydrogen	Nitrogen	0
Hydrogen	Formic acid	0
Nitrogen saturated with formic acid at 20° C.	Carbon dioxide	0
	Hydrogen	8·0

The experimental results shown in Table 1 suggest that both carbon dioxide and formic acid could scarcely be adsorbed on the catalyst surface and only hydrogen could be adsorbed almost in adsorption equilibrium.

The reaction was also studied in a closed circulating system. It is shown by this experiment and also by Topley and Hinshelwood[7] that the decomposition of formic acid on palladium is first order in formic acid and slightly retarded by hydrogen, being independent of carbon dioxide pressure.

If the adsorption on the whole surface is assumed to be similar to that on the active part, it seems reasonable to suppose from all of these results that the rate-controlling step in the formic acid decomposition would be the chemisorption of the acid on to the catalyst surface. The chemisorption rate of the acid on the catalyst surface is proportional to the partial pressure of the acid, while the sites available for the chemisorption is decreased by the increasing hydrogen adsorption as hydrogen pressure becomes higher, thus showing the retardation by hydrogen.

The reason why the apparent initial retention time of hydrogen is shorter during the decomposition than with inert carrier gas is supposedly due to the hydrogen produced by the decomposition decreasing the apparent adsorbability to hydrogen.

It has been shown thus far that we can study adsorption during surface catalysis with the gas chromatographic technique. Another example to be treated with this new approach is to study the adsorption of decomposing (or polymerizing) reactant on the catalyst. Suppose a decomposition (or polymerization) reaction of A, and that A is weakly adsorbed on the catalyst almost in adsorption equilibrium and the surface reaction is rate-determining. Using similar apparatus to that mentioned above, and with an inert carrier gas, the reactant is injected at the top of the adsorption column filled with catalyst. From the retention time of the remaining reactant at various temperatures, its heat of adsorption would be estimated.

Generally speaking, it is difficult to estimate the heat of adsorption of a decomposing (or polymerizing) reactant except from the adsorption measurements during catalysis as has previously been reported in the decomposition of formic acid on a silver catalyst[8], as neither the calorimetric method nor the isosteric heat method can be applied, since decomposition takes place simultaneously. The new method, however, will enable us to determine such heats of adsorption.

This work was carried out with financial support from Ashai Chemical Industry Promoting Foundation, Inc., Tokyo, and also from the Ministry of Education, to which my thanks are due. The assistance of Mr. H. Miyake is gratefully acknowledged. I also wish to thank Dr. R. Amemiya for helpful discussions.

Kenzi Tamaru

Department of Chemistry,
Yokohama National University,
Minami-ku, Yokohama.

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Polarography of Aromatic Hydrocarbons

Half-wave potentials of aromatic hydrocarbons have been plotted graphically against calculated energies of the lowest unoccupied molecular orbitals, localization energies of one or two positions in the molecule and spectroscopic data[1-6]. Various linear relationships were discovered during these attempts to correlate reduction potential with some fundamental quantity related to molecular structure.

In his contribution[6] Given stated that those authors who compared half-wave potential with the localization energy of two positions in the molecule did not understand properly the polarographic evidence[4,7-11], which he believed to indicate that only one electron was added in the potential-determining stage of reduction.

While a two-electron reduction, which aromatic hydrocarbons undergo, may proceed perhaps in two one-electron stages a strong argument can be made that the potential-determining stage encompasses the addition of two electrons. In view of such an argument, which follows here, is it wise to discourage comparisons of half-wave potential with appropriate quantities related to two-electron addition ?

Inspection of the plots of E versus $\log_{10} i/(i_d - i)$ for the polarographic waves of aromatic hydrocarbons dissolved in a variety of solvent mixtures[4,7-11] shows that near linearity exists. The polarographic waves are therefore described by the Kolthoff–Lingane[12] empirical equation:

$$E = E_{1/2} - (2{\cdot}303\ RT/\alpha\ nF) \log_{10} i/(i_d - i) \quad (1)$$

where E is the cathode potential, $E_{1/2}$ the half-wave potential, R the gas constant, T the absolute temperature, F Faraday's constant, i the current, i_d the limiting current, n the number of electrons calculated directly from the magnitude of i_d and α a parameter. For the aromatic hydrocarbons $n = 2$ and α is usually less than 0·5.

In the example of the aromatic hydrocarbons, a possible reaction sequence for the aqueous polarographic reduction of the C=C bond is:

$$\text{C=C} + 2e \underset{}{\overset{k_1}{\rightleftharpoons}} \overline{\text{C}}\text{—}\overline{\text{C}} \xrightarrow[\text{H}_2\text{O}]{k} \text{CH—}\overline{\text{C}} \xrightarrow[\text{H}_2\text{O}]{} \text{CH—CH} \quad (2)$$

The rapid and irreversible conversion of the dianion into the saturated hydrocarbon removes the dianion from prolonged electrode equilibrium with the unsaturated hydrocarbon. (Such dianions are only stable in the absence of proton donors[13,14].) An equation (3) relevant to the sequence (2) may be derived using the methods described in ref. 15 for an electron uptake succeeded by a rapid reaction:

$$-E = (RT/\alpha\ nF) \log_e 1{\cdot}23\ D^{1/2}t^{-1/2}\mu^{-1}k^{-1} + (RT/\alpha\ nF) \log_e i/(i_d - i) \quad (3)$$

where D is the diffusion coefficient of the unsaturated hydrocarbon, t the drop time, μ the reaction layer thickness, k_1 the rate constant for electron uptake and α is interpreted now as the fraction of potential which assists the forward reaction.

Equation (3) is seen to be similar in form to equation (1) so far as the variables E, i, n and α are concerned. The term $(RT/\alpha\ nF) \log_e 1{\cdot}23\ D^{1/2}t^{-1/2}\mu^{-1}k^{-1}$ is constant for a given medium, temperature and drop time, and therefore may replace $E_{1/2}$ in equation (1). Complete verification awaits experimental determination of μ and k_1.

The similarity of equation (3) to the empirical equation for the polarographic waves of aromatic hydrocarbons (equation 1) shows that their polarographic reduction is described adequately by a mechanism the potential-determining stage of which is due to uptake of two electrons.

H. J. Gardner

Chemical Research Laboratories,
Commonwealth Scientific and
Industrial Research Organization,
Box 4331 G.P.O., Melbourne. Nov. 21.

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BIOCHEMISTRY and PHYSIOLOGY

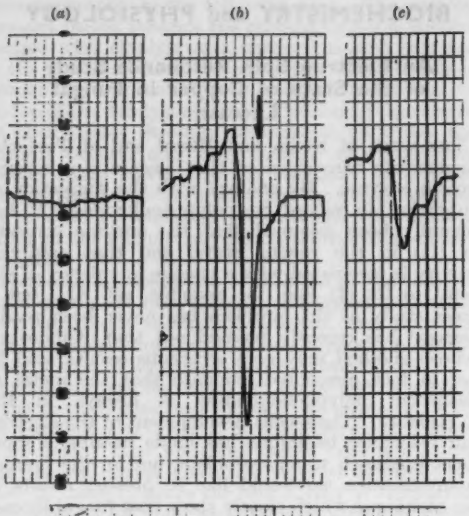
An Electron Spin Resonance Study of the State of Copper in Fungal Laccase

The work of Keilin and Mann[1] and of Tissières[2] conclusively demonstrated that laccase is a copper-protein enzyme. By analogy with the suggestion of Kubowitz[3] in regard to polyphenoloxidase, it has generally been assumed that the enzyme contains Cu (II) in the resting state, and that catalytic activity is associated with a change in valency of the metal to Cu (I) (see, for example, ref. 4); but a direct study of this problem has never been made. Kertész[5] has recently pointed out that Kubowitz's indirect method may give erroneous results, and he has, in fact, presented evidence that the metal in mushroom polyphenoloxidase is always in the Cu (I) state. The recent development of the electron spin resonance technique has made possible a more direct approach to this problem, and in the present communication we would like to present results of this technique which clearly show that fungal laccase contains Cu (II) which, during the oxidation of substrate, changes valency to Cu (I).

The fungal laccase used for this investigation was obtained as an induced exoenzyme of *Polyporus versicolor*; it was purified by a procedure recently described[6]. On a protein basis, the purified enzyme contains 0·4 per cent copper[6]. It is deep blue in colour with an absorption maximum at 615 mμ. However, the crude enzyme is often green, in which cases pigment with an absorption maximum at 405 mμ can be removed by various methods, for example, chromatography on 'Dowex 2' or on calcium phosphate (R. Mosbach, unpublished experiments). No way has been found to dissociate the blue colour from the enzyme.

For the measurements of electron spin resonance, 0·2 ml. of an approximately 2 per cent laccase solution in deionized water (*p*H about 5) was used. The samples were contained in quartz tubes having inside and outside diameters of about 3 and 4·5 mm., respectively. Substrates and other substances tested were added as solids, and the solutions were quickly frozen in liquid air. No attempt was made to exclude oxygen from the solutions. The electron spin resonance spectra were recorded with the frozen solutions at approximately 100° K. with a 3-cm. Varian *V*-4500 spectrometer.

Typical electron spin resonance absorption curves under a number of experimental conditions are given in Fig. 1. The blank in Fig. 1*a* shows that the quartz tubes and solvent used are essentially free of contaminating copper, while the strong resonance line shown in Fig. 1*b* demonstrates that the enzyme contains Cu (II) in the resting state, since Cu (I) is not paramagnetic and, consequently, would give no electron spin resonance signal. The absorption curve is asymmetric, probably due to an anisotropic g-factor. The g-value at maximum absorption, which should be close to $g_\perp$, is $2{\cdot}06 \pm 0{\cdot}01$; other covalent Cu (II) complexes have been found to have similar g-values[7]. The small peaks to the left of the centre seem to be associated with the copper resonance, since they disappear on reduction (Fig. 1*c*), and they probably originate from an anisotropic hyperfine structure. It is hoped that a detailed study of simpler Cu (II) complexes (mainly with amino-acids and peptides) now in progress may yield information



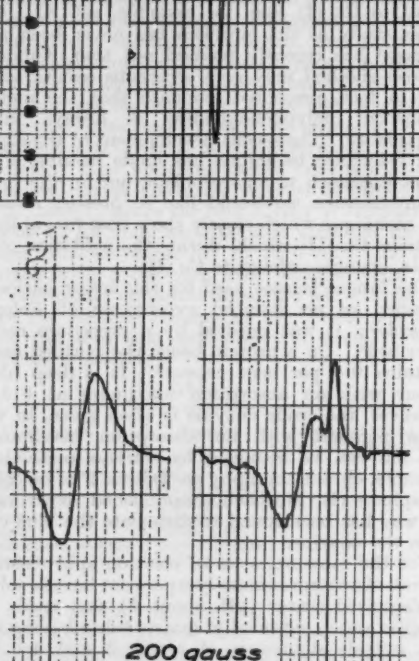


Fig. 1. Derivatives of electron spin resonance absorption curves recorded at about 100° K. Samples: (*a*) blank (deionized water); (*b*) about 2 per cent laccase solution; (*c*) about 2 per cent laccase solution plus excess catechol; (*d*) $1 \cdot 5 \times 10^{-3}$ *M* copper sulphate; (*e*) $1 \cdot 5 \times 10^{-3}$ *M* copper sulphate plus catechol. The arrow in (*b*) indicates the resonance field for free electrons

permitting a more exact interpretation of the laccase spectrum.

The absorption curve shown in Fig. 1*c* was recorded after a substrate (catechol) had been added to the enzyme solution used for Fig. 1*b*. The presence of substrate is seen greatly to diminish the electron spin resonance signal, showing that much of the metal has been reduced to Cu (I). Whether the remaining signal represents the steady-state concentration of oxidized enzyme, or is due to copper that is not associated with the activity of the enzyme, cannot be decided at present, since no experiments in the absence of oxygen have been performed. Other substrates, for example, ascorbic acid, cause a similar reduction in the resonance line, while, for example, tyrosine (a substrate of polyphenoloxidase) has no effect. When the substrate has been exhausted, the resonance reappears completely. It may be of interest to note that the blue colour disappears and reappears at the same time as the Cu (II) resonance line, though this does not necessarily mean that the colour is due to the metal ion (cf. refs. 8 and 9).

The spectra shown in Figs. 1*d* and 1*e* are included to demonstrate that the observed reduction in the resonance line cannot be due simply to complex formation with the substrate. Fig. 1*d* shows the absorption curve obtained with a solution of copper sulphate, and Fig. 1*e* that of the same solution after addition of catechol. It can be seen that, though the presence of substrate changes the shape of the absorption curve, it has no effect on the intensity of the signal.

The results reported here indicate that while Kubowitz's hypothesis[3] may not be valid for the enzyme for which it was formulated[5], it does appear to explain the role of the metal in laccase action. However, until more extensive experiments, in conjunction with kinetic measurements, have been performed, it is not possible unequivocally to relate the observed changes with the catalytic action of the enzyme. We hope to publish a more detailed report later.

This investigation has been supported by the Swedish Natural Science Research Council and the Rockefeller Foundation. We wish to thank Profs. A. Tiselius and K. Siegbahn for stimulating interest, and Dr. G. Fåhraeus for supplying the crude laccase from fungal cultures.

Bo G. Malmström
Rolf Mosbach
Tore Vänngård

Institutes of Biochemistry and Physics,
University of Uppsala. Nov. 7.

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Reaction of Sodium Borohydride with Wool

Recent developments[1-4] in the fractional extraction of wool proteins followed by their purification and physico-chemical characterization have led to a better understanding of the molecular structure of the fibre. But so far less than 50 per cent of the fibre has been separated into single proteins, and further work needs to be done in this field. A difficulty has been the lack of ideal methods for dissolving wool, for all those in current use suffer from one or more disadvantages, such as the side-reactions associated with the peracetic acid–ammonia procedure or the comparatively drastic conditions of *p*H and temperature often used with thioglycollate.

It has recently been found that sodium borohydride in alkaline solution rapidly dissolves wool under conditions of temperature and *p*H milder than many in current use. The reaction has been studied as follows: 1 gm. of solvent-scoured Merino wool was thoroughly wetted with 100 ml. of sodium borohydride solution, agitated at intervals and at the end of the reaction the residue filtered on to tared Whatman 54 filter paper, washed once with distilled water and dried at 105° C. to constant weight. The effects of

Table 1. Effect of Concentration and Temperature on the Extraction of Wool Protein by Sodium Borohydride

Sodium borohydride concentration (*M*)	Urea concentration (*M*)	*p*H		Temperature (° C.)	Time (hr.)	Wool dissolved (per cent)
		Start	Finish			
0·1	0	11·0	10·6	40	1	89
1·0	0	11·0	10·6	1	12	60
1·0	0	10·2	10·3	1	12	71
1·0	0	11·0	10·6	20	1	92
1·0	6	10·9	10·6	1	12	87
1·0	6	10·9	10·6	20	1	91

variation in reagent composition and reaction temperature on the extent of wool solution are shown in Table 1.

The influence of *p*H on the reaction was next studied; but was difficult to measure because of the buffering effect of the metaborate end-product and the increase in the decomposition rate of the sodium borohydride as the *p*H was lowered. However, some experimental results (Table 2) with 1·0 *M* sodium borohydride solutions at 1·0° C. suggest that wool was dissolved optimally at initial *p*H values in the range 10–11.

Table 2. Effect of Initial *p*H on the Extraction of Wool Protein by Sodium Borohydride at 1·0° C. for 12 hr.

Initial *p*H	Final *p*H	Wool dissolved (per cent)
9·1	10·5	35
10·2	10·3	71
11·0	10·5	52
11·8	11·5	7

The S-carboxymethyl derivative of the soluble protein was prepared by adjusting the *p*H of a digest solution (equivalent to 92 per cent of wool) to *p*H 8·5, a very slight excess over the theoretical amount of sodium iodoacetate was then added and the *p*H maintained at 8·5 for 15 min. The solution was then dialysed against running distilled water in a cellulose sausage casing with one end open to allow hydrogen to escape. The protein was then fractionated[2] on the basis of solubility at *p*H 4·5, the major part being insoluble under these conditions. Ultracentrifuge runs made on the *p*H 4·5 insoluble fraction, in 0·2 *M* potassium chloride at *p*H 7, showed the presence of at least 2 components (Fig. 1) of $S_{20\,w}$ 1·7 and 3·5 respectively, at a protein concentration of 1 per cent. Electrophoresis at *p*H 11 in 0·1 ionic strength buffer gave one spread-out peak in both boundaries.

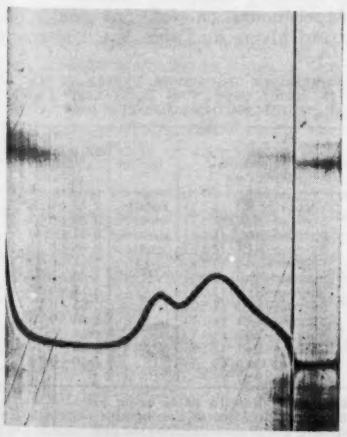

Fig. 1. Ultracentrifuge pattern of extracted wool protein (S-carboxymethyl derivative)

It is planned to study further the proteins in these extracts and in particular those more difficultly extractable materials which are largely undissolved by treatment of wool with alkaline thioglycollate solutions.

J. M. Gillespie*

Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Mass.
Nov. 18.

* Present address: Commonwealth Scientific and Industrial Research Organization, Division of Protein Chemistry, 343 Royal Parade, Parkville, Victoria, Australia.

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Preparation of Ceruloplasmin

Ceruloplasmin is one of the minor components of plasma proteins. Most of the numerous publications which have appeared in the past several years are concerned with the evaluation and clinical significance of hypo- and hyper-ceruloplasminæmia. Nevertheless, the biological role of ceruloplasmin is still unknown.

Clinical and laboratory research might be facilitated if ceruloplasmin could be available in sufficient quantities.

Ceruloplasmin was first isolated by Holmberg and Laurell[1] using a technique based on the precipitation of globulins by 50 per cent saturation with ammonium sulphate and subsequent denaturation of the accompanying proteins by repeated treatment with a mixture of alcohol and chloroform.

As normal plasma contains but 150–300 mgm./l. of ceruloplasmin, it would be necessary to combine the preparation of suitable quantities of ceruloplasmin with one of the routine fractionation techniques.

The handling of Holmberg and Laurell's alcohol–chloroform mixture is rather inconvenient for the technical preparation of ceruloplasmin. So, we investigated other methods allowing the subfractionation of fractions containing ceruloplasmin.

Rivanol, an acridine derivative, has been used for preparing γ-globulin[2] and more recently for the isolation of β_1-metal-combining globulin[3]. Neither γ-globulin nor β_1-metal-combining protein is precipitated by rivanol, and these proteins are separated by alcohol precipitation of the supernatant. We think that rivanol might be used as a more general separating agent if it should be possible to dissociate the rivanol–protein complex without damaging the protein. Ceruloplasmin is particularly indicated for such a study as this protein exhibits oxidase activity allowing an easy control of each step of the separation technique.

Ceruloplasmin has a strong tendency to interact with other proteins, and under the conditions of our routine work we find the copper-protein in fraction III and IV. We have succeeded in isolating ceruloplasmin of both these fractions by subfractionation with rivanol. The method is based on the following principles: (1) Precipitation by rivanol of some accompanying globulins at *p*H 6. No oxidase activity

should be detected in this precipitate. (2) Adjustment of *p*H to 7·8, and addition of another portion of diamino-ethoxyacridine-lactate in order to precipitate the fraction containing ceruloplasmin. Final *p*H should be 7·3–7·5. No oxidase activity should remain in the supernatant liquid. The latter might be used for preparing the β_1-metal-combining globulin in the case of fraction IV as starting material. (3) The rivanol–protein complex of step 2 is dissociated at *p*H 4; the accompanying proteins are eliminated by treatment with 50 per cent alcohol at *p*H 4·0. (4) The precipitate is extracted at *p*H 6. If further purification is necessary, it can be precipitated once more at *p*H 5·9, and 25 per cent alcohol concentration; it is then clarified before lyophilization.

M. Steinbuch
M. Quentin

Centre National de Transfusion Sanguine,
Paris.
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Influence of Ions on the Uptake of Glucose and on the Effect of Insulin on it by Rat Diaphragm

Insulin causes an increased uptake of glucose by the rat diaphragm *in vitro*[1]. The present communication reports the results of some experiments designed to study the influence of physiologically important cations on the uptake of glucose by the diaphragm of the rat in the presence and absence of insulin. Incidentally, the effects of the other related monovalent metallic ions and of changes in gas phase were also studied.

The incubation mixture consisted of a suitable buffer in isotonic sucrose or in a mixture of isotonic sucrose and isotonic solutions of the chlorides of certain mono- and di-valent metals. In every instance the *p*H of the medium was adjusted to 7·4 and glucose added to make a final concentration of about 250 mgm./100 ml. The buffers used, and their concentrations in the medium, were as follows: lithium, sodium and potassium bicarbonates, 0·02 *M*, disodium hydrogen phosphate, 0·01 *M*, *tris*aminomethane, 0·025 *M*. One hemidiaphragm was suspended in 1 ml. of the medium containing, where indicated, 100 milliunits of insulin per ml. The incubation was carried out at 38° C. for 90 min.

The results given in Table 1 show that diaphragm incubated in isotonic sucrose containing no metallic ion (*tris* buffer) or only small amounts of them (bicarbonate or phosphate buffer) removed glucose from the medium in amounts comparable to those removed from Gey and Gey's salt mixture. Insulin, however, had little or no effect on the uptake of glucose under such conditions. The ability of the tissue to respond to insulin appeared only when rather high concentrations of metallic ions such as sodium, potassium, rubidium, cæsium and magnesium were present in the medium. There was, however, little or no response to insulin in the presence of varying amounts of lithium or calcium.

The poorness of the response to insulin in media containing lithium ions apparently resulted from the high basal uptake of glucose from such media. On the other hand, the absence of any insulin response

Table 1. Effect of Metallic Ions on the Influence of Insulin in causing Increased Uptake of Glucose by Rat Diaphragm under Aerobic Condition

Medium	Buffer	Glucose uptake (mgm./hr./gm. of tissue) Without insulin	With insulin (100 milli-units/ml.)	*P*
Gey and Gey's salt mixture	$NaHCO_3$	3·16±0·14	7·67±0·44*	<0·001
Sucrose	$NaHCO_3$	4·15±0·15	4·18±0·21	>0·05†
Sucrose	$KHCO_3$	4·11±0·28	4·64±0·26	<0·01
Sucrose	$LiHCO_3$	6·49±0·20	6·61±0·21	>0·05
Sucrose	Na_2HPO_4	4·58±0·19	4·87±0·15	>0·05
Sucrose	*Tris*	4·43±0·22	4·68±0·15	>0·05
Sucrose–NaCl (1 : 1)	$NaHCO_3$	5·16±0·28	8·04±0·40	<0·001
Sucrose–NaCl (1 : 1)	Na_2HPO_4	3·84±0·30	6·12±0·27	<0·001
Sucrose–NaCl (1 : 1)	*Tris*	4·46±0·12	7·18±0·06	<0·001
Sucrose–KCl (1 : 1)	$KHCO_3$	2·82±0·22	4·81±0·17	<0·01
Sucrose–KCl (3 : 1)	$KHCO_3$	2·99±0·25	5·59±0·18	<0·001
Sucrose—$MgCl_2$ (2 : 1)	*Tris*	3·84±0·13	5·79±0·39	<0·01
Sucrose–$CaCl_2$ (2 : 1)	*Tris*	0·61±0·13	0·71±0·08	>0·05
Sucrose–LiCl (1 : 1)	$LiHCO_3$	7·95±0·31	9·10±0·31	<0·01
Sucrose–LiCl (1 : 1)	*Tris*	6·80±0·24	7·93±0·40	<0·02
Sucrose–RbCl (2 : 1)	*Tris*	3·24±0·21	5·52±0·33	<0·001
Sucrose–CsCl (2 : 1)	*Tris*	3·30±0·13	6·33±0·37	<0·01

P = probability that the difference is due to chance.
* Standard error of the mean.
† Not significant.

in the presence of calcium ions was possibly due to their toxic effects, as evidenced by the very low basal uptake of glucose in the presence of calcium ions. In both cases, the basal uptake of glucose could be adjusted to about the normal level by reducing the amounts of lithium or calcium ions added; but then again there was little or no response to insulin, presumably because the concentrations of the essential ions in the media were too low.

It has already been noted that the basal uptake of glucose in the presence of lithium ions was high. In fact, the uptake differed in a regular way in the presence of equivalent amounts of the different metallic ions in the medium. A comparison of the uptake of glucose by the diaphragm in the presence of equivalent amounts of the monovalent metallic ions in the medium is given in Table 2. It is seen that the uptake followed the order of lithium > sodium > potassium = rubidium > cæsium. It is interesting to note that this is the order of the above ions in the so-called lyotropic or Hofmeister series.

The experiments so far reported were all carried out under aerobic condition. The results of a few similar experiments carried out under anaerobic condition are given in Table 3. The results show

Table 2. Comparison of Glucose Uptake by Rat Diaphragm incubated in Media containing Equivalent Amounts of the Monovalent Metallic Ions

Experiment No.	Composition of medium	Buffer	Glucose uptake	*P*
1	Sucrose–LiCl (1 : 1)	Bicarbonate	7·48±0·26*	<0·05†
	Sucrose–NaCl (1 : 1)		5·83±0·43	
2	Sucrose–NaCl (1 : 1)	Bicarbonate	5·74±0·37	<0·01
	Sucrose–KCl (1 : 1)		3·08±0·18	
3	Sucrose–LiCl (1 : 1)	*Tris*	7·37±0·45	<0·001
	Sucrose–NaCl (1 : 1)		4·26±0·27	
4	Sucrose–NaCl (1 : 1)	*Tris*	4·14±0·27	<0·01
	Sucrose–KCl (1 : 1)		2·55±0·21	
5	Sucrose–KCl (1 : 1)	*Tris*	2·30±0·14	>0·05‡
	Sucrose–RbCl (1 : 1)		2·44±0·16	
6	Sucrose–KCl (1 : 1)	*Tris*	2·05±0·16	<0·02
	Sucrose–CsCl (1 : 1)		1·56±0·10	

Experiments were done in pairs using hemidiaphragm from the same rat and comparing two of the metallic chlorides in turn under aerobic condition. *P* = probability that the difference is due to chance.
* Standard error of the mean.
† Significant.
‡ Not significant.

Table 3. Influence of Insulin on Glucose Uptake by Rat Diaphragm incubated in Mixtures of Isotonic Sucrose and Isotonic Chlorides of Sodium and Potassium under Anaerobic Condition

Medium	Buffer	Glucose uptake (mgm./hr./gm. of tissue) Without insulin	With insulin (100 milli-units/ml.)	P
Sucrose–NaCl (1 : 1)	$NaHCO_3$	7·91 ± 0·22	8·39 ± 0·25*	>0·05†
Sucrose–NaCl (1 : 1)	Na_2HPO_4	3·32 ± 0·12	3·52 ± 0·12	>0·05
Sucrose–NaCl (1 : 1)	*Tris*	2·52 ± 0·09	2·81 ± 0·07	<0·02
Sucrose–KCl (1 : 1)	$KHCO_3$	3·93 ± 0·25	4·40 ± 0·21	<0·05‡

P = probability that the difference is due to chance.
* Standard error of the mean.
† Not significant.
‡ Significant.

that under anaerobiosis there was little response to insulin even when the medium contained the required ions in sufficient amounts. It may, however, be noted that with bicarbonate buffer the basal uptake of glucose tended to be higher under anaerobic conditions than under aerobic conditions. This was particularly true for sucrose–sodium chloride medium. These observations agree with those on the effect of anaerobiosis in increasing the basal uptake of glucose by rat diaphragm from Gey and Gey's salt mixture previously reported by Randle[2] from this laboratory.

From a consideration of the foregoing results one wonders whether there are two separate and distinct processes (one, insulin-independent and the other, insulin-dependent) involved in the uptake of glucose by the diaphragm of the rat. The process independent of insulin, although markedly influenced by strong ionic environments, can, nevertheless, function in a more or less normal way in the absence of metallic ions in the added medium. It can also function both under aerobic and anaerobic conditions. The process dependent on insulin, however, seems to operate best under aerobic condition and in the presence of rather high concentrations of certain metallic ions, including the physiologically important ions of sodium, potassium and magnesium. Ions of sodium and potassium are actually present in large amounts in the extracellular and intracellular fluids, respectively. The results reported here may thus have some physiological significance with respect to insulin action in the whole animal. They may also throw some light on the now generally accepted glucose-transport theory of insulin action[3].

The effect of the chloride ion, which was present in the medium in large amounts in most of the experiments reported here, has not been considered specifically. A general consideration of the results, however, indicates that most probably the chloride ion was not responsible for the effects of the added salts.

I am indebted to Prof. F. G. Young for advice and criticism and for the grant of laboratory facilities. My thanks are due to the managers of the I.C.I. Fellowships Fund, University of Cambridge, for the grant of a fellowship. The sample of crystalline insulin used in the above experiments was kindly given by Dr. C. C. G. Pope of the Wellcome Foundation, London.

Gangagobinda Bhattacharya

Department of Biochemistry,
University of Cambridge.

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Immunochemically Active Cross-linked Polystyrene

The communication of Gyenes, Rose and Sehon[1] prompts us to record briefly some observations in a closely related field.

For some time a group of our colleagues has been investigating the separation of antigens by means of their immunochemical specificity[2]. They have, for example, attempted to invert the method used by Campbell[3] and others for the fractionation of antibodies, and, by chemically conjugating antibody protein to a polymer such as cellulose, have used the resulting product in a column to separate antigens. The amount of antibody protein which can be incorporated in this manner is not large, and as a result of the generally unfavourable stoichiometry in respect to the antigen in the immune reaction, the capacity for the antigen is quite low. It occurred to us that greater capacity could be achieved if the antibody protein could be made an integral part of the three-dimensional structure, and we proceeded independently on lines precisely similar to those of Gyenes, Rose and Sehon.

'Distrene', an essentially linear polymer of styrene, was nitrated, initially with fuming nitric acid/sulphuric acid to give a product containing approximately 1 nitro group per styrene residue, and the product reduced with a large excess of stannous chloride to the polyamino compound[4]. This was soluble in dilute mineral acid but insoluble in dilute alkali. On diazotization in acid solution it gave, surprisingly, a very sparingly soluble diazonium salt. The γ-globulin fractions (which are rich in antibody) from several immune sera, prepared against a partially purified protein fraction from *Pasteurella pestis* (rabbit), against *B. anthracis* (horse) and an anti-ovalbumin serum (rabbit) were prepared by sodium sulphate precipitation[5]. The suspension of the polydiazonium styrene was then coupled with the immune γ-globulin in phosphate buffer at *p*H 8·5 and the excess unreacted diazonium groups rendered unreactive by coupling with resorcinol. The resulting cross-linked polymers were washed thoroughly with buffer until the filtrates were free from protein and were stored in isotonic saline. They contained 20–30 per cent of their weight of firmly bound protein. In view of the insolubility in water of the polydiazonium styrene, it was more closely examined and found to contain a small percentage of sulphonic acid groups, derived presumably from the sulphuric acid used in the nitrating process. When this was omitted, the product after reduction gave a completely soluble diazonium salt which would conjugate with much more γ-globulin. After treatment as above, the final polymer contained 60–70 per cent of its weight of protein. The products, though softer than the normal cross-linked polystyrene resins, are easily handled and readily filterable.

These 'resins' show approximately the capacity for absorbing their respective antigens that would be expected from the neutralizing capacity of the anti-sera from which they are prepared. They do this in all cases with a marked degree of specificity. In some cases the specificity is high. Thus, a 'resin' prepared from an anti-anthrax serum removed the protective antigen quantitatively from a culture filtrate prepared by the method of Thorne and Belton[6] without significant reduction in the total protein content of the filtrate. A 'resin' prepared from an anti-ovalbumin serum failed to remove

bovine serum albumin from buffer solution but quantitatively removed ovalbumin under the same conditions: that from the plague anti-serum, though it removed the toxin quantitatively under all conditions tested, also removed some other protein. As several other antigens are present in the protein fraction used as antigen and the anti-serum contains antibodies to several constituents of the plague organism, this is not surprising.

The preparations were subject to a more stringent test for specificity by comparing their behaviour with precisely similar 'resins' prepared from normal γ-globulin. In the case of the anti-ovalbumin 'resin', the same high specificity was shown. Under the same conditions that the anti-ovalbumin 'resin' removed ovalbumin quantitatively from solution the resin prepared from normal rabbit γ-globulin removed none. The latter 'resin' did, however, remove some protein from the protein fraction from *P. pestis* and, despite a number of attempts, we have not succeeded in reducing this non-specific absorption below 15–25 per cent in this case.

We have also succeeded in preparing cross-linked resins by direct mercuration of polystyrene and cross-linking the polymer chains via the sulphydryl group of the antibody protein. Their immunochemical properties are exactly similar to those of the diazo 'resins'. Anthrax antigen absorbed on the anti-anthrax 'resin' when injected into rabbits and guinea pigs confers active immunity, and the animals will survive the challenge of a lethal dose of anthrax organisms. This indicates that the antigen retains its biological activity when absorbed.

Full details of this work will be published elsewhere.

We are indebted to Dr. D. A. L. Davies and Dr. A. P. MacLennan for toxicity tests in mice and to Mr. F. C. Belton for immunological assays of the anti-anthrax preparations.

L. H. Kent
J. H. R. Slade

Microbiological Research Establishment,
Ministry of Supply,
Porton, Nr. Salisbury, Wilts.

Nov. 24.

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Evidence for the Conversion of Quinic Acid to Shikimic Acid in Roses

The simultaneous occurrence of quinic and shikimic acids has been reported in several plants[1–3]. No definite role has been ascribed to either of these alicyclic acids in higher plants, except for participation of the former in the synthesis of chlorogenic acid[4].

In certain micro-organisms, shikimic acid has been demonstrated to be in the direct path to aromatic ring biosynthesis[5]. Microbial response to exogenous quinic acid has been reported in secondary mutants of *Aerobacter* which grew well when supplied with quinic acid[5]. Similar results have been reported for a mutant of *Neurospora*. A quinic dehydrogenase, which converts quinic acid to 5-dehydroquinic acid, has been demonstrated in *Aerobacter*[5], although the enzyme is not widely distributed. Davis[5] has concluded that quinic acid is not directly involved in aromatic ring biosynthesis in micro-organisms, and that where a requirement has been shown to exist, it is due to an adventitious quinic dehydrogenase.

The recent discovery of a high concentration of quinic acid in rose tissues[6] suggested the use of this plant for the biosynthesis of quinic acid labelled with carbon-14. This was accomplished by exposing actively growing plants of the variety 'Redbird' to $^{14}CO_2$ in light. Quinic acid labelled with carbon-14 was isolated by ion-exchange chromatography and crystallized. The labelled acid was fed into young blooms of 'Better Times' roses through cut stems and allowed to metabolize for 5, 23, 45 and 71 hr. before harvesting. The blooms were fractionated and radioactivity was determined in each of the non-volatile organic acids, free amino-acids, sugars and protein amino-acids.

More than 25 per cent of the carbon-14 in quinic acid fed to the roses could not be accounted for in any fraction and was presumably metabolized and lost as $^{14}CO_2$. This was confirmed in a subsequent experiment, where it was found that about 20 per cent of the radioactivity was evolved as $^{14}CO_2$ in 71 hr.

In petals, approximately 10 per cent of the incorporated carbon-14 could be accounted for in other compounds, and the remainder was present as quinic acid. About 25 per cent of the carbon-14 appearing in non-volatile organic acids was recovered as labelled shikimic acid. In petals of roses harvested after 71 hr., about 20 per cent of the carbon-14 in free amino-acids appeared in tyrosine and about 20 per cent appeared in phenylalanine.

The results show that quinic acid is metabolized by roses. This is borne out by other results indicating that quinic acid normally increases in concentration in rose blooms until they become about half open, after which the concentration decreases[7]. A similar development has been observed in apple fruits[3]. The evidence is clear that roses contain the enzymes necessary to reduce quinic to shikimic acid, although no evidence can be presented as to whether or not it follows the classical mechanism via quinic dehydrogenase, 5-dehydroquinase, and 5-dehydroshikimic reductase[5]. The possibility that the label in shikimic acid could arise from decarboxylation of quinic acid and its subsequent incorporation via a dark fixation mechanism has been ruled out since earlier experiments have shown no label to be incorporated into the quinic–shikimic fraction during dark fixation of $^{14}CO_2$.

A complete report of this work will be published in the *Contributions from Boyce Thompson Institute.*

L. H. Weinstein
C. A. Porter
H. J. Laurencot, jun.

Boyce Thompson Institute for
Plant Research, Inc.,
Yonkers, New York.

Nov. 20.

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Co-existence of the Embryonic (Third Normal) Hæmoglobin Fraction with Erythroblastosis in the Blood of Two Full-term New-born Babies with Multiple Malformations

In previous communications[1,2] the existence of a third normal, embryonic hæmoglobin was reported to be present in the blood of fœtuses up to five months of intra-uterine life. The hæmolysates of the blood of these fœtuses were characterized by their electrophoretic mobility on filter paper in a buffer solution of *p*H 8·6, which was slower than that shown by the hæmolysates obtained from the cord blood of full-term new-borns. The paper electrophoresis method did not permit us, however, to separate the different hæmoglobin fractions present either in the fœtal or in the embryonic blood.

By using the column chromatographic procedure described by Huisman and Prins[3], with some minor modifications, we were able to separate the three distinct fractions from the hæmolysate of a four month old embryo weighing 90 gm.

The hæmolysates of the cord blood of full-term new-born babies which were used at the same time as controls showed only two fractions (Fig. 1). The purpose of this communication is to report the finding of the third (embryonic) hæmoglobin fraction in the blood of two full-term new-born babies with multiple congenital malformations.

A further interesting and unusual feature of the blood of these new-borns was an erythroblastosis which was found in the blood film and which could not be explained by a blood group incompatibility between mothers and infants.

One baby weighing 2,560 gm. was born to a healthy 28-year-old primipara and showed the following congenital malformations: 1, multiple defects of the scalp; 2, hare lip; 3, polydactyly of both hands; 4, cryptorchism.

Mother and infant had identical blood groups (A CDe/CDe). No hepatosplenomegaly was found. In the blood film an unusually large number of nucleated red cells in all stages of maturation was found, but their absolute number was not determined. 1 hr. after birth the infant died. The hæmolysate of the cord blood of this malformed infant was found to contain 66·9 per cent of the alkali-resistant type of hæmoglobin. Paper electrophoresis did not reveal the presence of embryonic hæmoglobin, whereas the column chromatographic method, using an 'Amberlite *ICR* 50' column and a citrate buffer solution of *p*H 6·0 with a sodium ion concentration of 0·15 as an eluting solvent at a rate of flow of 9 ml. per hr., did reveal the three distinct hæmoglobin fractions similar to those shown in Fig. 1.

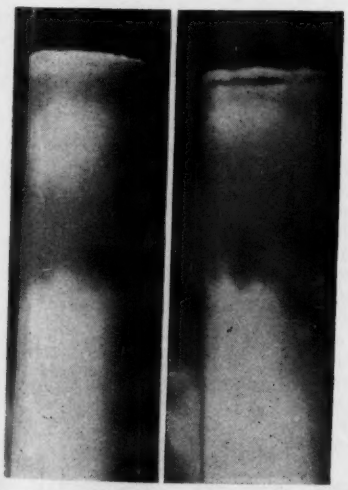

Fig. 1 'Amberlite' column chromatography of (left) the hæmolysate of the cord blood of a full-term new-born showing two hæmoglobin fractions (adult and fœtal) and (right) of the hæmolysate of a four-months-old embryo showing three hæmoglobin fractions (adult, fœtal and embryonic)

Another baby was born to a healthy mother of two healthy children. The malformations of this child consisted of: 1, fusion of the two nasal fossa into one cavity; 2, under-development of the external genital organs of male type; 3, absence of the gonads in the very small scrotum and in both inguinal canals. The infant died shortly after birth, and section revealed the presence of a uterus and of two gonads, which on histological examination proved to be ovaries. The blood groups of both parents and of the child were identical (O cDe/cDe). The blood count of the baby's peripheral blood showed: hæmoglobin 18·2 gm./100 ml.; red blood count 6·4 millions/cu. mm.; white blood count 25,000/cu. mm. Examination of the blood film showed: nucleated erythrocytes, 45/100 white blood count. The direct and indirect Coombs tests were negative. The hæmolysate of the cord blood of this baby contained 73·3 per cent of the alkali-resistant hæmoglobin fraction, and column chromatographic examination revealed three distinct hæmoglobin fractions.

The use of the 'Amberlite' column chromatographic procedure represents an important step forward in the separation of the different fractions of normal hæmoglobin. Thus, we were able to separate not only the two hæmoglobin fractions known to be present in the cord blood of full-term new-borns, but also the third, embryonic, fraction (the lowest in the chromatographic column) in the blood of small embryos.

The interest of the present observation lies in the fact that by using the column chromatographic method we were, for the first time, able to discover the embryonic hæmoglobin fraction in the blood of two full-term new-borns who were severely malformed, whereas twenty other normal new-borns who served as controls did not show this fraction in their blood.

Another interesting fact lies in the co-existence of the embryonic hæmoglobin fraction with erythroblastosis in the blood of these malformed infants.

Erythroblastosis fœtalis has hitherto been found either associated with blood-group incompatibilities of the Rh and ABO systems or occasionally in the blood of prematures or of infants born to diabetic mothers. So far, we are unaware of reports on erythroblastosis in the blood of full-term new-borns with congenital malformations, in the absence of the above-mentioned conditions.

It is generally admitted that the synthesis of the normal, as well as of the abnormal, hæmoglobins is genetically determined, and so is the physiological process of replacement of the fœtal by the adult type of hæmoglobin which starts very early in embryonic life and continues until its completion between the sixth and the twenty-fourth month after birth.

It seems equally probable that the synthesis of the embryonic hæmoglobin as well as its replacement by the later (fœtal and adult) hæmoglobins are determined by genetic factors. The persistence of the

embryonic hæmoglobin in the blood of the two above-described malformed new-borns could therefore be explained by a disturbance of one or more genetic factors which leads to the appearance of multiple developmental mutations, one of them being the persistence of embryonic hæmoglobin.

The association of persistent embryonic hæmoglobin with erythroblastosis in the blood of the new-borns is another phenomenon which remains to be explained by further investigations.

I. Halbrecht
Department of Obstetrics and Gynecology,
Hasharon Hospital,
Ch. Klibanski
F. Bar Ilan
Medical Research Center, Beilinson Hospital,
Petah Tikva, Israel. Nov. 5.

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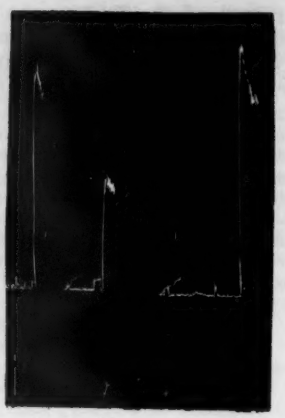
Pharmacological Actions of γ-Butyrobetaine

γ-Butyrobetaine is a substance which has been known for many years to be present in the tissues of some cold-blooded animals, for example, snakes (*Python*)[1] and freshwater eels[2]. It appears, however, not to be present normally in mammalian tissues, but it has been located in muscle extract[3] and decaying horse flesh[4], and may appear in the urine in phosphorous poisoning[5] or in pernicious anæmia[6]. Linneweh[7] has shown that γ-butyrobetaine injected into dogs is largely excreted unchanged, although a small proportion is oxidized to carnitine.

Recently, Hosein[8] has shown that γ-butyrobetaine can be identified in the brains of mammals in which convulsions have been induced by the intraperitoneal injection of the so-called cerebral convulsants, while it is never found in this form in the brains of normal animals. Other studies by Hosein[9] have revealed that in normal animals γ-butyrobetaine occurs as a complex with coenzyme *A*. The pharmacological actions of γ-butyrobetaine, after subcutaneous administration into mice, were reported by Linneweh[7]. He found that there was increased respiration, profuse salivation and lacrymation, dilatation of the pupils, vasoconstriction, voiding of urine and fæces, block of motor nerve endings and cardiac arrest in diastole. A fatal dose of γ-butyrobetaine as the chloride was 70–80 mgm. in a 15-gm. mouse. From these observations Linneweh concluded that γ-butyrobetaine behaved as a curare-like substance.

In the light of Hosein's findings, we have felt it desirable to investigate again some of the pharmacological actions of this substance. γ-Butyrobetaine was prepared synthetically as the free base and used for all tests. Subcutaneous injection of as little as 1 mgm. was found to be fatal to a 25-gm. mouse, and the sequence of events described by Linneweh[7] was invariably observed. There is evidently a profound autonomic excitation which culminates in death apparently due to cardiac failure.

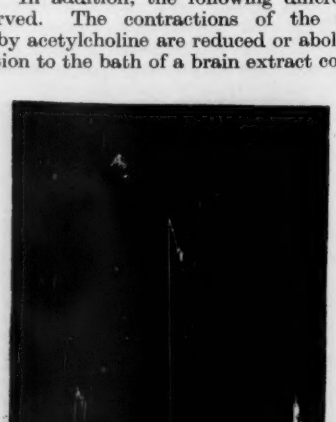
In its actions γ-butyrobetaine resembles those of acetylcholine except that it is much less active on a weight-for-weight basis and its action is considerably prolonged. It seems not to be enzymatically destroyed in the body at any appreciable rate, as Linneweh's experiments on its recovery from urine showed. We have observed a parallelism in action between γ-butyrobetaine and acetylcholine for depression of the blood pressure of the cat, stimulation of the perfused superior cervical ganglion in the cat, and inhibition of the isolated perfused frog heart. Inhibition of mammalian neuromuscular transmission, similar to that caused by large doses of acetylcholine[9], is invariably found.



(*a*) (*b*) (*c*)

Fig. 1. The effect of acetylcholine and factor I on the isolated ileum of the guinea pig. (*a*) Acetylcholine, $1 \cdot 2 \times 10^{-9}$; (*b*) acetylcholine, $1 \cdot 2 \times 10^{-9}$ + factor I, 0·003 units/ml. of bath fluid; (*c*) acetylcholine, $1 \cdot 2 \times 10^{-8}$

In only one preparation has it been possible to show a qualitative difference in action between γ-butyrobetaine and acetylcholine. The isolated ileum of the guinea pig is induced to contract by the addition of γ-butyrobetaine to the bath. There is an initial quick response, resembling that caused by acetylcholine, which is sometimes followed by a slow tonic contraction which may continue for several minutes. In addition, the following difference can be observed. The contractions of the intestine induced by acetylcholine are reduced or abolished by the addition to the bath of a brain extract containing



(*a*) (*b*) (*c*)

Fig. 2. The effect of γ-butyrobetaine and factor I on the isolated ileum of the guinea pig. (*a*) γ-Butyrobetaine, 5×10^{-4}; (*b*) γ-butyrobetaine, 5×10^{-4} + factor I, 0·003 units/ml. of bath fluid; (*c*) γ-butyrobetaine, 5×10^{-4}

an inhibitory principle (factor I)[10]. This effect is shown in Fig. 1. However, when γ-butyrobetaine and factor I are added together to the intestine bath, there is an enhancement of the contraction (Fig. 2). No evidence of an inhibitory effect of factor I on the contractions induced by γ-butyrobetaine similar to that observed with acetylcholine has ever been found. In those cases where a slow tonic contraction of the tissue succeeds the quick response, it appears that the potentiation due to factor I is confined to the fast phase[10].

Thus, although the parallelism in action between γ-butyrobetaine and acetylcholine is close, it is not entirely general. Since the relationship between acetylcholine and factor I on the ileum of the guinea pig is not entirely clear, it is not possible to comment on the difference here observed with γ-butyrobetaine. The other pharmacological actions of γ-butyrobetaine can be satisfactorily explained by a prolonged action like that of acetylcholine.

E. A. Hosein

Department of Biochemistry,
McGill University, Montreal.

H. McLennan

Department of Physiology,
University of British Columbia,
Vancouver.

Nov. 12.

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Content of Deoxyribonucleic Acid of the Germinal Vesicle of the Primary Oocyte in the Rabbit

By means of centrifugation experiments, evidence of persistence of deoxyribonucleic acid in the growing nucleus of the primary oocyte was put forward by J. Brachet[1]. J. Govaert[2] was able to demonstrate that, in *Fasciola hepatica*, the acid not only persists but also remains quantitatively constant in the germinal vesicle. The same quantitative constancy was later found by J. Mulnard[3] to be existing in the oocytes of *Acanthoscelides obtectus*.

During our studies on content of deoxyribonucleic acid in the ovary of the rabbit, we tried to discover if this same constancy also occurs in this mammalian species. In the rabbit ovary, fixed in alcohol (75 parts), formalin (20 parts) and acetic acid (5 parts), the Feulgen reaction shows more or less visible chromosomal loops in the germinal vesicles of the oocytes, present in primary follicles (Figs. 1 and 2). This distribution, although not favourable, permits measurements by means of L. Lison's histophotometrical method[4], so that a relative amount of deoxyribonucleic acid, expressed in arbitrary units, can be obtained and compared with a standard value, given by the mean acid content of spermatocytes I measured in a rabbit testis under identical experimental conditions. The mean content of deoxyribonucleic acid for 107 measured oocytes was $2{,}400 \pm 50{\cdot}2$ ($S = 523{\cdot}7$), while for 301 spermatocytes I we obtained $2{,}393 \pm 26{\cdot}8$ ($S = 465{\cdot}4$).

Figs. 1 and 2. Demonstration of the youngest (1) and oldest (2) measured germinal vesicles, stained by the Feulgen reaction. Statistical computation shows that, so far as their content of deoxyribonucleic acid is concerned, the nuclei between those two limits belong to the same population (*n*, nucleolus, surrounded by a ring of nucleolus-associated chromatin)

The distribution of deoxyribonucleic acid found in those young germinal vesicles seemed to permit only the demonstration of an order of magnitude.

These results suggest that in the rabbit oocyte I, the content of deoxyribonucleic acid corresponds to the theoretically expected content ($4n$, as in the spermatocytes I). Moreover, this content remains unchanged at least during the formation of the young germinal vesicle, up to the stage measured here.

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D. Van de Kerckhove

Department of Normal and Comparative Anatomy,
Belgian Centre of Normal and Pathological Growth,
University of Ghent.

Nov. 13.

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Liver Hexokinase Activity in Rats adapted to Intermittent Starvation

Fábry's experiments[1] which were performed in this laboratory have shown that, in rats adapted to intermittent starvation, more glycogen is deposited in the liver after the intake of an equal amount of a mixed diet than in rats fed normally. We may thus expect that in these animals there develops an adaptation of the enzymes participating in glycogen synthesis. We have therefore attempted to estimate the liver hexokinase activity, because this enzyme has a key position in the synthesis of glycogen.

We used female Wistar rats with an initial weight of about 100 gm. During the experiment the animals were kept at a temperature of 21–23° C. During the six-week period of adaptation to intermittent starvation we interposed gradually longer fasts between the days when the animals had free access to food. During the last week of adaptation the animals were fed only twice[1]. The total food consumption in the adapted animals amounted to 60 per cent of the intake of control animals fed *ad lib.* Throughout the experimental period the animals had free access to water. The rats were fed on a stock diet (Larsen's diet) providing about 25 cal. per cent protein, 25 cal. per cent fat and 50 cal. per cent carbohydrate.

Table 1. Hexokinase Activity of Liver Homogenate of Rats adapted to Intermittent Starvation

Experimental group	No. of rats	Glucose utilized ($\gamma \pm$ S.E.)	P	Initial glycogen content ($\gamma \pm$ S.E.)	Uptake of glycogen ($\gamma \pm$ S.E.)
A. Stock diet					
Fed animals: adapted	8	25·5 ± 8·1	< 0·05	256 ± 78	75 ± 19·5
controls	6	2·9 ± 8·5		215 ± 62	55 ± 13·5
Fasted for 24 hr.: adapted	8	30·0 ± 6·0	< 0·02	34 ± 5·1	28·9 ± 7·0
controls	7	10·5 ± 6·2		23 ± 3·8	20·0 ± 3·6
Fasted for 48 hr.: adapted	7	27·0 ± 3·7	< 0·01	32·5 ± 3·9	31·6 ± 2·1
controls	6	8·5 ± 3·0		26·6 ± 6·5	23·2 ± 5·7
B. Carbohydrate-free diet					
Fasted for 24 hr.: adapted	6	10·5 ± 3·5	N.S.	21·2 ± 2·7	14·5 ± 3·2
controls	6	9·5 ± 4·4		24·2 ± 5·3	19·5 ± 3·4

The amount of homogenate used corresponded to 20 mgm. liver (wet weight). 150γ glucose was added.

Hexokinase activity was estimated in the liver homogenate, using Long's method[2] based on the disappearance of added glucose. Simultaneously, we investigated the glycogen content at the beginning and at the end of a period of incubation in trichloracetic deproteinate, using Nelson's method[3]. Because the nitrogen content of the liver, calculated on a wet-weight basis, is practically the same in animals adapted to intermittent starvation and in control animals[4], we evaluated the results on a wet-weight basis.

Enzyme activity was assessed in animals fed 10 gm. of Larsen's diet per 100 gm. body-weight (the food was consumed freely overnight) and after a 24- and a 48-hr. fast respectively. Before fasting, the rats received a weighed quantity of Larsen's diet (10 gm./100 gm. body-weight).

As can be seen from Table 1, rats adapted to intermittent starvation have a markedly increased hexokinase activity, both when satiated and while fasting, compared with normally fed controls. The uptake of added glucose, which is used as the criterion of hexokinase activity, was on average 20 per cent in these animals, while in rats fed normally it was considerably lower, even approaching zero. The small uptake of glucose in the liver homogenate of normal rats has already been recorded[2,5].

The relatively high content of glycogen in this tissue and the glycogenolysis during incubation obviously play a part in this phenomenon. In animals adapted to intermittent starvation, the glycogen content of the liver homogenate is equal to or somewhat higher than in the controls; the glycogenolysis during incubation could thus only diminish the difference between the adapted animals and controls, and have an adverse effect on our results.

In animals adapted to intermittent starvation, a marked hyperphagia develops when they have access to food[4] and the intestinal absorption of glucose is increased[7]. It is therefore possible that the increased activity of liver hexokinase was induced by the intermittently increased supply of glucose by the portal blood to the liver. We therefore adapted a further group of animals to intermittent starvation, using the same method. The Larsen stock diet was, however, replaced by a diet practically free of carbohydrate (69·9 cal. per cent protein, 29·75 cal. per cent fat and 0·75 cal. per cent carbohydrate). Despite the fact that in adapted animals even under these conditions hyperphagia developed when the animals were allowed food, we found no difference in the hexokinase activity of adapted and control animals fed *ad lib.* on the same diet after a 24-hr. fast (Table 1*B*). We can thus assume that the deciding factor for the increase of liver hexokinase, revealed in the intermittently starving animals on a mixed diet, was the intermittently increased supply of glucose absorbed by the liver during the periods of free access to food. We thus may speak of a "substrate-induced enzymatic adaptation"[8].

R. Petrášek

Physiological Department,
Institute of Human Nutrition,
Budějovická 800,
Prague 14.
Oct. 16.

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Granulocyte Precipitins of Human Tissues and Activated Sera

In a preliminary communication[1] we reported that the addition of water-soluble extracts of human granulocytes to saline extracts of any other human cell resulted in the formation of a precipitate. This phenomenon can be studied by Ouchterlony's double gel diffusion method[2]. The substances giving rise to the precipitate were designated as 'granulocyte factor' and 'tissue factor'.

In the present communication it will be shown that the tissue factor exists in most human sera, including those from apparently normal subjects, but in an inactive form. Activation of this substance can be accomplished by dialysis of the sera against physiological saline.

The methods used to prepare extracts from granulocytes and other leucocytes will be presented in detail elsewhere. Human granulocytes were isolated from heparinized blood of patients with chronic myelogenous leukæmia using polyvinylpyrrolidone according to Wasastjerna's procedure[3], modified to handle large amounts of blood. Contaminating erythrocytes were removed by the addition of hypotonic saline, which lysed the red cells but not the leucocytes. The thoroughly washed cells were homogenized in ice-cold saline in a Waring blendor, and the supernate was dialysed against distilled water. After removal of the precipitate, the water-soluble fraction was lyophilized. Extracts of normal leukocytes and white cells from other forms of leukæmia were obtained in a similar manner.

The double gel diffusion method has been described[4]. All cups were filled simultaneously with 0·2 ml. of the reagents and refilled the next day with the same amount. When the extracts were used at optimal concentration, lines of precipitate could be observed within 24 hr. From Fig. 1 it can be seen that the

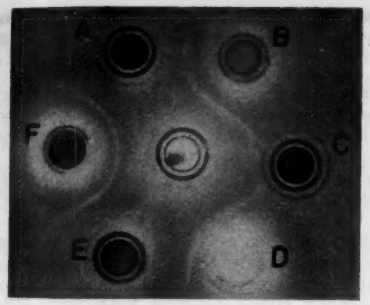

Fig. 1. Centre cup and cups *A* and *E*, extracts from granulocytes of three different patients with chronic myelogenous leukæmia. Cups *B* and *F*, extracts of leucocytes from two patients with acute leukæmia. Cup *D*, extract of leucocytes from a patient with chronic lymphocytic leukæmia. Cup *C* is empty

extracts of granulocytes did not react with each other, but that lines of precipitate were formed between the extracts of granulocytes and other leucocytes. These lines simulated immune systems in that they were dissolved if the initial concentration of one of the reagents was in excess, and they migrated away from the cup containing this reagent. The reaction is specific in that only extracts from normal or leukæmic granulocytes react with the other cell extracts. It resembles in this respect the system described by Seligmann *et al.*[5]. The tissue factor has been demonstrated in all human cells examined, including those grown in tissue culture or on the allantoic membrane of the chick embryo, indicating that it is not a serum contaminant.

No reactions were observed between extracts of granulocytes and untreated human sera. However, after dialysis against saline, most of these sera reacted in the same manner as did extracts from cells containing the tissue factor (Fig. 2). When activated sera were placed adjacent to a cup containing tissue extract, the two resulting lines of precipitate coalesced, indicating that the precipitin of the serum was the same as that of the tissues.

The fact that most human sera could be activated by dialysis suggests that they contain a substance of low molecular weight capable of inhibiting the reaction between serum tissue factor and the granulocyte factor. It is not known whether the tissue factor has a physiological function, but the presence of an inhibitor against it indicates that it may. At present we are trying to isolate and define the two precipitating factors as well as their inhibitor. This system is now also being studied in pathological sera.

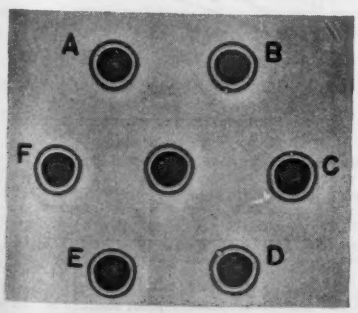

Fig. 2. Centre cup, extract of granulocytes; Cups *A*, *C* and *E*, dialysed sera (1 : 2). Cups *B*, *D* and *F*, the same sera before dialysis

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Leonhard Korngold
Gerda Van Leeuwen
Daniel G. Miller

Division of Experimental Pathology,
Sloan-Kettering Institute for Cancer Research,
and the Sloan-Kettering Division,
Cornell University Medical College,
New York.

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Comparison of the Formation of the Complex, Sensitized Erythrocyte-Complement ($EAC'_{1,4,2}$) in Hæmolytic Systems differing in their Hæmolysin and Complement-fixing Activities

In a recent study[1] we compared the complement fixing and hæmolytic titres of several different hæmolytic systems. We found a lack of correlation between the titres of these two activities when compared in different hæmolytic systems. In addition, we found striking differences in the quantity of complement (C') capable of being fixed by these systems. These findings indicated that C' fixation and hæmolysis represent quantitatively distinct serological properties.

The sequence of action of the C' components has been determined by Mayer and co-workers[2] for the sensitized sheep cell. The demonstration of this sequence, as well as current work[3,4] concerned with the participation of the C' components in immune hæmolysis, involves the fixation of measurable amounts of the C' components by sensitized cells (EA). In view of our findings we thought it would be of interest to compare the fixation of the C' components in hæmolytic systems which differ markedly in their C'-fixing activities.

As illustrated by Mayer *et al.*[2], in the Forssman antibody and sheep cell system, $EAC'_{1,4,2}$ formation may proceed at 0° C. without undergoing appreciable lysis. They have further shown that the presence of ethylenediamine tetraacetic acid prevents formation of the $EAC'_{1,4,2}$ complex by binding calcium and magnesium ions which are necessary for the formation of this complex. Employing this information, we examined the extent of $EAC'_{1,4,2}$ formation in three different hæmolytic systems according to the following procedure.

1 ml. of a serial two-fold dilution of each antiserum was placed in two series of tubes in an ice-bath. 1 ml. of a 1 : 10 dilution of whole guinea pig *C*, and 1·0 ml. of the standardized cell suspension were added. The tubes were incubated for 4 hr. at 0° C. with frequent mixing. Then 1·0-ml. amounts of 0·05 *M* ethylenediamine tetraacetic acid was added to one of each series of tubes to give a final concentration of 0·01 *M* in a total reaction volume of 5·0 ml. All tubes were then incubated for 30 min. at 37° C., centrifuged and the amount of lysis was determined by examining the supernatants spectro-

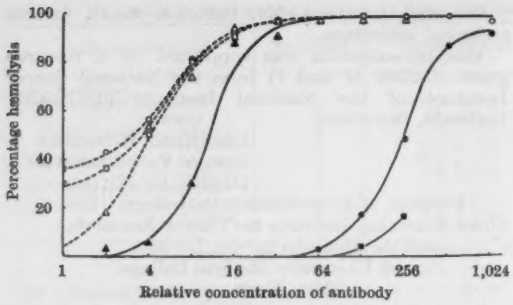

Fig. 1. The comparison of $EAC'_{1,4,2}$ formation in three hæmolytic systems. Circles, rabbit anti-sheep and sheep cells; squares, human anti-A and sheep cells; triangles, rabbit anti-human O and O cells. Open symbols, in the absence of ethylenediamine tetraacetic acid; solid symbols, in the presence of ethylenediamine tetraacetic acid

photometrically. Included were appropriate controls for: spontaneous lysis, the extent of lysis occurring during incubation at 0° C., and the effectiveness of $C'_{1,4,2}$ fixation inhibition by ethylenediamine tetraacetic acid.

The results are presented in Fig. 1 as a plot of per cent lysis versus relative antibody concentration. Antibody concentrations were examined in each system which gave approximately the same amount of lysis in the absence of ethylenediamine tetraacetic acid. This was done so that with ethylenediamine tetraacetic acid added, the relative amounts of antibody required for lysis in each system could be directly compared. It can be seen that, although the relative amounts of antibody in each system gave equivalent lysis in the absence of ethylenediamine tetraacetic acid, the relative amounts of antibody required for lysis in the presence of ethylenediamine tetraacetic acid were strikingly different for each system. Twice the concentration of antibody was required in the rabbit anti-human system for sufficient $EAC'_{1,4,2}$ complex formation at 0° C. to obtain lysis equivalent to that which was obtained in the absence of ethylenediamine tetraacetic acid. The rabbit anti-sheep and human anti-A serums with sheep cells required approximately 60 and 95 times respectively the amount of antibody to obtain equivalent lysis in the presence of ethylenediamine tetraacetic acid. These findings correspond well with the ratios of the hæmolysin and C'-fixing titres previously obtained for these systems[1].

These results illustrate differences in the ability of these hæmolytic systems to form the $EAC'_{1,4,2}$ complex at 0° C., as judged by the relative amounts of antibody required for lysis with the addition of ethylenediamine tetraacetic acid. These results further show that the hæmolysin titres in the presence of ethylenediamine tetraacetic acid are unrelated to the hæmolysin titre in the absence of ethylenediamine tetraacetic acid. However, the hæmolysin titres in the presence of ethylenediamine tetraacetic acid do show a relationship to the C'-fixing activities of these systems.

Harold S. Goodman
Mount Sinai Medical Research Foundation,
Chicago.

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Effect of Cross-linking by Formaldehyde on the Contraction–Relaxation of Rat Tail Tendon

Recently, Banga *et al.*[1] demonstrated that tendons of the tail of a rat spontaneously shortened and relaxed when they were placed in 40 per cent potassium iodide at room temperature. Aging influenced the length–time curves so that rapid contraction was followed by rapid relaxation in the young but slow relaxation in the old. Cross-linking in collagen has been a widely discussed concept of the effect of the aging process. If the development of more highly polymerized collagen is the effect of aging then tanning young tendons with formaldehyde, a cross-linking reaction, should 'age' them a certain amount. The following experiments were done to test this hypothesis.

Tendons of the tail of a rat, having mass/length ratios of 0·3–0·4 mgm./cm., were treated for 15 min. by exposing them to various dilutions of 37 per cent formalin in sodium acetate solutions buffered at *p*H 7·8. Following treatment, 6-cm. lengths were clamped inside a glass cylinder where the bottom end of the fibre was fixed and the top was free to actuate the direct writing arm of an electrically driven kymograph. A mechanical magnification of × 5 was obtained for the curvilinear co-ordinate system. Plots were made in rectilinear co-ordinates of length as a function of time for each tendon studied. The net force on the tendon was estimated at between 1 and 2 gm. but was constant throughout the study. Chemical contraction was induced at 23° C. by using freshly prepared aqueous 40 per cent potassium iodide, which was added rapidly to the tube containing the fibre.

Fig. 1 shows that the length–time curves depend upon the concentration of formaldehyde used for cross-linking collagen in the tendons. At 1/1,000 dilution, the fibres contracted 65 per cent of their length in less than 10 min. and remained so for at least 50 min. At 1/100,000 dilution there was a 40 per cent decrease in length in less than 5 min. which returned to the original length 40 min. later. These measurements were repeated using 0·5 *M* acetic acid instead of 40 per cent potassium iodide, and the same response was obtained to formaldehyde treatment.

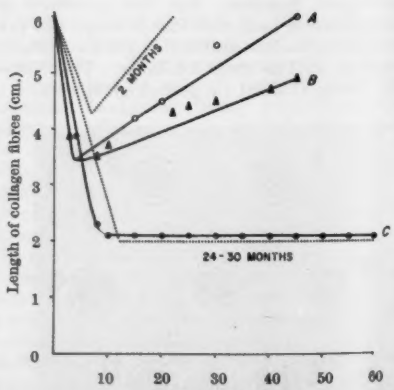

Fig. 1. The contraction–relaxation of tendons of the rat tail induced by 40 per cent potassium iodide as a function of degree of cross-linking by formaldehyde. *A*, 1 : 10,0000 formalin; *B*, 1 : 10,000 formalin; *C*, 1 : 1,000 formalin; – – – –, untreated collagen fibres; ——, formalin-treated fibres

The reaction of aldehydes on collagen has been studied many times. It is generally accepted that at alkaline *p*H the terminal amino-group of lysine in the collagen reacts with formaldehyde to form a part of the cross-link[2]. Solomons and Irving[3] have shown that there is a direct relationship between the degree of mineralization in bone and the amount of bound lysine and hydroxylysine. The solubilization of hide collagen by concentrated acetic acid solutions also involved the lysine residue as shown by Elden *et al.*[4] from spectroscopic studies of collagen–acetic acid–formaldehyde interactions. In view of the similarity between the effect of aging and formaldehyde cross-linking on the length–time curves of tendon we suggest that collagen lysine and hydroxylysine might be involved in the changes taking place.

Wiederhorn *et al.*[5] cross-linked kangaroo tail tendon with formaldehyde and measured its elasticity after thermal shrinkage. The stress required for a fixed elongation was greater after the treatment. According to Flory[6], the force required to produce a fixed amount of strain must be higher with more cross-linking. At a fixed amount of force the elongation would be less. In the tendon of the kangaroo there was an increase in cross-linking with a reduction in the molecular weight between bridge points from 55,000 to 15,000.

The thermal shrinkage of collagen has features which suggest that it is a true phase-transition[7]. Solubilization by dilute acetic acid resembles the first step in a recently proposed two-step mechanism for the thermal conversion of the crystalline region of collagen to an amorphous phase. Contraction of tendons in 0·5 *M* acetic acid and 40 per cent potassium iodide is the mechanical consequence of such transitions. The thermally induced phase transition does not produce viscous-flow characteristics. Therefore, relaxation cannot proceed because of irreversible slipping of melted crystallites.

To sum up the above comments we have the following facts: (1) aging increases the extent of contraction but decreases relaxation of rat tail tendons. (2) Introducing cross-links by formaldehyde treatment affects the length–time curves in a manner comparable to the aging process. (3) The action of 40 per cent potassium iodide and acetic acid (0·05 *M*) are such as to induce a phase change with concomitant shortening of the tendons. Relaxation diminishes with aging. Under a fixed load the length of 'elastic' collagen decreases (contraction increases) when further cross-links are introduced. (4) Relaxation is due to the partial dissolution of the tendon by the solvent allowing the chains of collagen to unfold.

These facts are consistent with the concept that an increase in cross-linking of collagen occurs with aging.

H. R. Elden

Howard Hughes Medical Institute,
Miami, Florida.
Nov. 25.

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Electrical Conductance and Dielectric Constant of the Interior of Erythrocytes

The electrical conductivity of an electrolyte is determined by its ion concentration. The effect of dispersed solid particles of a comparatively low conductance, such as proteins, can be taken into account using formulæ given by Maxwell[1] and Fricke[2]. Hence, an analysis of the electrical conductivity of the cell interior should provide information about the organization of its protoplasm.

In 1912, Höber[3] reported specific resistance values of the interior of beef erythrocytes between 160 and 600 ohm cm. Fricke and Morse[4] gave for beef and sheep erythrocytes 310 ohm cm. and Fricke and Curtis[5] later on for sheep, rabbit, chicken and turtle 140 ohm cm. Rajewsky and Schwan[6] and Cook[7] quote values of 100–110 ohm cm. for human erythrocytes. Measurements of Li and Schwan[8] indicate values near 200 ohm cm. for beef erythrocytes. In order to clarify whether these discrepancies reflect true species differences or differences in methods, additional investigations with erythrocytes of several species were undertaken[9]. Fresh heparinized blood was tightly packed by centrifugation. The erythrocyte sediment contained 2–5 per cent trapped plasma as judged from the consistency of calculated internal data with different packing factors and from the established performance of the hæmatocrit centrifuge[10].

Conductivity $\varkappa$ and dielectric constant relative to free space ε were measured with an admittance bridge (*RX*-Meter 250-*A*) from 0·5 to 250 Mc.

The reactance of the cell membrane vanishes[11] at about 70 Mc. Hence, $\varkappa$ and ε of completely packed erythrocytes are identical with internal values of the erythrocytes above 70 Mc./s. This was checked by investigation of the low-frequency conductance of erythrocyte suspensions after destruction of the insulating membrane with either saponin or toluene. Measured values were corrected for the small amount of trapped plasma in order to obtain values characteristic of the interior. This was done by use of equations given by Fricke[2]. While the exact validity of these formulæ may be questionable, no significant error should result from this procedure.

Results are summarized in Table 1 for seven different species. The internal resistance between the different animals varies by not more than 15 per cent. Differences are significant within 3 per cent. However, the dielectric constants of the several species, accurate to 1 dielectric unit, are not significantly different. The average of all dielectric constants values is 52 and close to previously published values[6]. It is due to the hæmoglobin content of the cells corresponding to a dielectric decrement of about 0·7 unit per gm. hæmoglobin per 100 c.c.[11]. The internal conductivity cannot be readily explained by the known electrolyte content, even if an activity coefficient of a 0·2 *M* potassium chloride solution is assumed and the non-conducting volume of the hydrated hæmoglobin is considered. The calculated conductivity values are higher than the internal results reported above by a factor of 2. Partial binding of ions to protein and reduction of their mobility may provide explanation for this fact. Similar discrepancies are indicated in the case of liver cells[9,12], frog muscle[13], and nerve fibres[14]. This suggests that the lower conductance is a general property of cell plasm.

Table 1. Internal Specific Resistance ρ, Conductivity $\varkappa$ and Dielectric Constant ε of Red Blood Cells (Temperature, 25° C.)

Species	ρ [ohm cm.] 90 Mc./s.	$\varkappa$ [m.mho/cm.] 90 Mc./s.	ε (250 Mc./s.)
Man	193	5·18	50·1
Beef	230	4·35	51·3
Sheep	228	4·39	50·4
Dog	216	4·63	50·9
Cat	190	5·26	53·1
Rabbit	170	5·89	55·1
Chicken	204	4·90	52·1

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H. Pauly

Electromedical Division,
Moore School of Electrical Engineering,
and
Department of Physical Medicine,
School of Medicine,
University of Pennsylvania,
Philadelphia 4.

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Synthetic Activity of Polynucleotide Phosphorylase from Sperm

Polynucleotide phosphorylases are enzymes that catalyse the synthesis of polyribonucleotides.

No evidence has yet been given for the presence of polynucleotide phosphorylase in extracts of animal tissues, although these enzymes have been found in extracts of aerobic or anaerobic Gram-positive or Gram-negative bacterial cells[1–4].

A polynucleotide phosphorylase was isolated and partially purified from the human sperm. The enzyme was prepared from vacuum-dried human sperm by the method of Littauer and Kornberg[5]. The preparation obtained was further purified by ammonium sulphate fractionation, and by zinc ethanol precipitation. The precipitate obtained, between 15 and 20 per cent, was adsorbed on an equal volume of calcium phosphate gel. The pure enzyme was then eluted from the gel and activity determined.

The enzymic reactions were carried out in solutions where each 5·0 ml. portion contained *M*/10 trihydroxymethyl-amino-ethane buffer, *p*H 8·0, *M*/60 magnesium chloride, *M*/15 of either adenosine-5′-diphosphate, guanosine-5′-diphosphate, uridine-5′-diphosphate or cytidine-5′-diphosphate, each alone or in pairs, and 0·5 ml. of 5 per cent solution of the sperm enzyme.

Chromatograms of the reaction solutions were developed by the descending technique in two dimensions. The solvent systems used were ammonium–*iso*butyric acid and tertiary amyl alcohol–formic acid–water[6,7]. Permanent records of the chromatograms were obtained by the photographic technique[7]. The polynucleotides located were cut out from the chromatograms and eluted. The ultra-violet absorption spectrum, chemical identity and concentration were studied.

Table 1. Stoichiometry of Reactions Catalysed by Sperm Phosphorylase

	Nucleotides		Ortho-phosphate	Polynucleotides	
Initial	ADP	9·8	1·0	ADP	0·0
90 min.	ADP	−5·5	+6·0	ADP	+5·0
Initial	GDP	9·4	1·0	GDP	0·0
90 min.	GDP	−5·0	+6·0	GDP	+5·0
Initial	UDP	9·6	1·0	UDP	0·0
90 min.	UDP	−5·2	+6·0	UDP	+5·0
Initial	CDP	9·6	1·0	CDP	0·0
90 min.	CDP	−5·4	+6·0	CDP	+5·0
Initial	ADP+GDP	19·8	2·0	ADP . GDP	0·0
90 min.	ADP+GDP	−10·5	+12·0	ADP . GDP	+10·0
Initial	ADP+UDP	20·0	2·0	ADP . UDP	0·0
90 min.	ADP+UDP	−10·8	+12·0	ADP . UDP	+10·0
Initial	ADP+CDP	19·4	2·0	ADP . CDP	0·0
90 min.	ADP+CDP	−10·2	+12·0	ADP . CDP	+10·0
Initial	GDP+UDP	−19·0	2·0	GDP . UDP	0·0
90 min.	GDP+UDP	−10·0	+12·0	GDP . UDP	+10·0
Initial	GDP+CDP	19·0	2·0	GDP . CDP	0·0
90 min.	GDP+CDP	−10·6	+12·0	GDP . CDP	+10·0
Initial	UDP+CDP	19·9	2·0	UDP . CDP	0·0
90 min.	UDP+CDP	10·8	+12·0	UDP . CDP	+10·0

ADP, Adenosine-5′-; GDP, guanosine-5′-; UDP, uridine-5′-; CDP, cytidine-5′-phosphates.

Homopolymers of adenylic, uridylic, guanylic and cytidylic acid with several heteropolymers containing the adenylic-guanylic, adenylic–uridylic, adenylic–cytidylic, guanylic–uridylic, guanylic–cytidylic and uridylic–cytidylic are found in the above enzyme reaction solutions (see Table 1). The enzymic activities of the polynucleotide phosphorylase provide a possible explanation of the differences in composition and structure of ribonucleic acids.

Anwar A. Hakim

Biochemical Research Laboratories,
Miami Heart Institute,
4701 North Meridian Avenue,
Miami Beach 40, Florida.

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Influence of Vitamin D on Synthesis of Hexosamine by Rachitic Rat Cartilage

It is believed that chondroitin sulphate or its protein complex plays a very important part in the process of calcification[1]. The fact that during calcification there is a marked increase in metachromatic staining in the region which exhibits calcification led Rubin and Howard[2] to postulate that either there is an increase in the concentration of the mucopolysaccharide or that there is a change in its state of polymerization. In the former case it is possible that

the enzyme system responsible for synthesizing chondroitin sulphate or some of its components would show an increase during the healing of rickets brought about by vitamin D, just in the same manner as the citric acid content and the enzyme concerned with its formation were found to show an increase in the rachitic epiphyseal cartilage of rat and dog after vitamin D administration[3]. Castellani and Zambotti[4] have recently demonstrated that the epiphyseal cartilage of growing rabbit contained appreciable amounts of a thermolabile enzyme system which synthesizes one of the components of chondroitin sulphate, namely, hexosamine from glutamine and glucose-6-phosphate. In view of these considerations, it was felt desirable to investigate whether vitamin D influenced the enzymatic synthesis of hexosamine in the rachitic cartilage.

For this study young albino rats, four weeks old, were fed a rachitogenic diet[5] for two weeks. At the end of this period the rachitic animals were divided into two groups, taking litter and sex distribution into account. Animals in group 1 served as rachitic controls; those in the other were each administered a single dose of vitamin D (4,000 I.U.) 96 hr. earlier. The animals from both groups were killed on the same day, and cartilage from the distal end of femur and the proximal end of tibia was removed from the four rats in each group. The pieces of cartilage were pooled, weighed and ground in a chilled mortar with a known volume of a cold solution of 10^{-2} *M* glutamine and 10^{-2} *M* glucose-6-phosphate in 0·1 *M* citrate buffer (*p*H 6·4), and hexosamine formed enzymatically was determined as described by Castellani and Zambotti employing Schloss's method[6]. As shown in Table 1 there is an appreciable rise in the hexosamine-synthesizing capacity of the rachitic rat cartilage as a result of vitamin D administration.

Table 1. Effect of Vitamin D on the Enzymatic Formation of Hexosamine by Rachitic Rat Epiphyseal Cartilage

No. of observations	μgm. Glucosamine/gm. wet tissue in 3 hr.				
	Rachitic *A*	96 hr. after vitamin D *B*	$B-A$ / *S.E.*	*t*	*P*
5	267 (19)	459 (20)	192±38·5	4·97	<0·01

Number of rats employed is indicated in parentheses.

It was therefore logical to expect that on administration of vitamin D to rachitic animals the concentration of chondroitin sulphate or its hexosamine component in the cartilage would show an increase. This point was therefore investigated. Young rats were made rachitic as described above and distributed equally into four groups. Animals in group 1, which served as rachitic controls, were killed and their tibial epiphyseal cartilage quickly removed, placed individually in tubes and dried at 75° C. for 4 hr. Animals in the other groups were administered a single dose of vitamin D (4,000 I.U.), killed 48, 96 and 144 hr. later and the tibial cartilage was scooped out and dried. The dried cartilage was weighed and hydrolysed with 3·0 ml. 4 *N* hydrochloric acid at 100° C. for 12 hr. The hydrolysate was made to volume in a 10 ml. volumetric flask and aliquots taken for hexosamine determination. As shown in Table 2, there is a small, but significant, fall in the hexosamine content of rachitic rat cartilage during healing induced by vitamin D.

Table 2. Effect of Vitamin D on Hexosamine in μgm./mgm. Dry Rachitic Rat Cartilage

Rachitic controls	Hours after vitamin D treatment					
	48		96		144	
Group 1	2		3		4	
S.E.		*S.E.*		*S.E.*		*S.E.*
117·8±5·55	97·5±4·00		89·3±6·25		87·3±3·74	
	Between groups 1 and 2		Between groups 1 and 3		Between groups 1 and 4	
t	2·97		3·41		4·56	
P	<0·02		<0·01		<0·001	

It was indeed surprising that although vitamin D enhanced the capacity of the healing cartilage to synthesize hexosamine enzymatically, there was no commensurate increase in the hexosamine content of cartilage, which in fact showed a slight decrease. It is difficult to reconcile these two contradictory observations. Further work might throw light on this interesting problem.

P. K. Dikshit

Nutrition Research Laboratories,
Indian Council of Medical Research,
Coonoor, South India.
Nov. 18.

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Incorporation of Labelled Thymidine in the Seminal Vesicle of the Mouse

It has been reported that labelled adenine is incorporated into the deoxyribonucleic acid of the seminal vesicle of the mouse at a rate far exceeding that required for cell-division in the organ[1]. In view of the implications of this finding it seemed desirable to repeat the experiment with ^{3}H-thymidine, which can be regarded as a specific precursor for deoxyribonucleic acid[2]. The present communication deals with the incorporation in the seminal vesicle and, for comparison with a dividing tissue, in the œsophagus of the same animals.

Eleven mice of an inbred strain were injected intraperitoneally with 16·7 μc. each of ^{3}H-thymidine with a specific activity of 360 mc. per m*M*, supplied by Messrs. Schwarz Inc., Mount Vernon, N.Y. Animals were killed 1–7 days later, organs were fixed for 1 hr. in acetic acid–alcohol followed by 24 hr. in formol saline, embedded in paraffin wax and sectioned at 5–6μ thickness. Autoradiographs of unstained or Feulgen-stained sections were prepared by the stripping-film technique and exposed for 13 or 23 days. Unstained specimens were stained with Ehrlich's hæmatoxylin and eosin after photographic processing. The background of the autoradiographs is 0·3–0·5 grains per nucleus.

A number of nuclei of epithelial cells of the seminal vesicle are labelled 24 hr. after injection. The intensity of labelling after 13-days exposure averages 15 grains per positive nucleus in the seminal vesicle, compared with 13 grains per positive nucleus in the basal layer of the œsophagus. The most heavily labelled nuclei show up to 50 grains in both tissues.

Table 1. Comparison of Labelling of Deoxyribonucleic Acid and Cell Division in the Seminal Vesicle and Œsophagus of Mice

	SI (per cent)	*MI* (per cent)	$\frac{SI}{MI}$
Seminal vesicle	4·4 ± 0·9	0·05 ± 0·02	88
Œsophagus	18 ± 1·1	9·5 ± 1·0	1·9

SI, No. of labelled nuclei per 100 cells 24 hr. after injection of labelled thymidine.

MI, No. of metaphases per 100 cells after 6 hr. treatment with colchicine.

$\frac{SI}{MI}$, the ratio of labelled cells to dividing cells as scored under the experimental conditions in this investigation.

No corrections for absorption of β-particles or loss of material through sectioning of nuclei of different size have been made. Furthermore, it is by no means certain that the supply and dilution by the pool of unlabelled thymidine of the injected ^{3}H-thymidine are the same for œsophagus and seminal vesicle. It can, however, be concluded that those nuclei which incorporate labelled thymidine do so at approximately the same rate in both tissues, though differences may be masked by the sources of error mentioned.

The seminal vesicle of mice killed 24 hr. after injection contains 4·4 ± 0·9 per cent (Table 1) labelled nuclei while the basal layer of the œsophagus shows 18 per cent. Analysis of the movement of labelled nuclei in the Malpighian layer of the œsophagus indicates that the differentiating cells are replaced every 2–3 days[3], so that the relatively low figure of 18 per cent labelled nuclei indicates that labelled thymidine is used up 8–12 hr. after injection, in agreement with data recently published by Hughes *et al.*[4]

The number of nuclei incorporating ^{3}H-thymidine is thus four times higher in the basal layer of the œsophagus than in the seminal vesicle; the ratio of cell division should be similar in the two tissues if all incorporation were in preparation for mitosis. Allen[5], however, has found only 40 mitoses per 100,000 cells after 6 hr. treatment with colchicine, equivalent to 160 divisions per 100,000 cells per day or an average life-span of approximately 600 days. Mitotic counts on sections adjacent to those used for autoradiography and on Feulgen-stained sections of mice killed 6 hr. after injection with colchicine agreed with Allen's data (Table 1). No signs of cell degeneration such as pycnosis or extrusion of cells into the secretion were observed. Measurements of the amount of deoxyribonucleic acid per nucleus by means of Feulgen-photometry (Richards, B. M., private communication) in polymorphs and nuclei of the seminal vesicle gave a narrow range of diploid values. No intermediate or polyploid values were found.

Incorporation of ^{3}H-thymidine into the deoxyribonucleic acid in the seminal vesicle of the mouse is therefore approximately forty times higher than could be expected from the known rates of proliferation in this organ. This excess is far beyond the limits of error of the techniques employed.

Depending on the length of time during which thymidine is available, the rate of incorporation in 4·4 per cent of the nuclei of the seminal vesicle is equivalent to a renewal of 4·4–13 per cent of its deoxyribonucleic acid per day. From the rate of incorporation of labelled adenine a renewal of 10 per cent per day was calculated[1]; the two methods therefore agree as well as can be expected.

The proportion of labelled nuclei is well maintained up to seven days after injection. Cell counts and grain counts undertaken so far suggest a decline between the third and seventh day, which, however, may not be significant in view of the small number of animals used in this investigation.

Since most of the incorporation of ^{3}H-thymidine in the epithelial cells of the seminal vesicle does not lead to mitosis a metabolic function of this process has to be assumed. Metabolic activity of deoxyribonucleic acid was originally discovered by means of the incorporation of labelled phosphate in rat liver by Ahlstrom, v. Euler and Hevesy[6]. The results of later experiments which give similar results have been reviewed in a previous communication[1]. They lead to the conclusion that deoxyribonucleic acid is metabolically almost completely stable in adult brain, cartilage and muscle, that it is renewed at a rate approaching 1 per cent per day in normal rat liver and pancreas, and proceeds at a rate similar to that found in dividing tissues in Jensen rat sarcoma, transplantable mouse mammary carcinoma, lung, intestinal mucosa, mouse prostrates grown in organ culture and in the seminal vesicle of the mouse.

It is of interest to note that our findings, similar to recent observations on incorporation of labelled thymidine in bean roots[7], suggest that all, or at least most, of the deoxyribonucleic acid contained in nuclei is taking part in metabolic activity which therefore is unlikely to be connected with genetic specificity of deoxyribonucleic acid.

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S. R. Pelc
P. B. Gahan

Medical Research Council
Biophysics Research Unit,
King's College, London, W.C.2.
Nov. 4.

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Terminology of Hormones

A need has been felt for a precise terminology to distinguish four types of animal hormones by their actions[1,2]. (1) 'Metabolic' hormones stimulate or inhibit clearly defined metabolic processes. This term is in general use for such hormones as thyroxine and a secretion from the insect corpus allatum, both of which increase oxygen consumption, or for the hydrocortisone-like hormones from the adrenal cortex and a neuro-secretion from the insect brain, both of which increase diuresis. (2) 'Morphogenetic' hormones promote growth and cell differentiation. This term can be used for thyroxine when it has a morphogenetic action in stimulating amphibian metamorphosis. The hormone, ecdysone, from the prothoracic gland of some insects is also morphogenetic, since it promotes moulting and ecdysis. There is obviously some overlap between the hormones in these two groups, since no growth can occur without the support of many metabolic processes; but metabolic actions can occur without growth and can usually be examined and described independently. The distinction in terms can help to clarify this.

(3) These hormones control the relatively rapid and repeatable actions of effector organs. They form a very distinct group, for which we propose the term 'kinetic', since no other name has been generally accepted. They include oxytocin and other hormones acting on muscles, chromactivating substances from the ganglionic X-organ–sinus gland complex of crustaceans acting on retinal cells and chromatophores and secretin among those controlling exocrine glands. They have been referred to as energetic[2]; but this is not a term that distinguishes them clearly from metabolic hormones. Great confusion surrounds the use of the suffixes trophic or tropic not only for some of these kinetic hormones, as in chromatophorotropic, but also for some of the morphogenetic hormones.

(4) Those kinetic hormones which stimulate the secretion of another hormone from an endocrine gland can be usefully distinguished by the rational, if not euphonious, term 'endocrinokinetic'. This includes hormones from the vertebrate adenohypophysis, such as thyrotrophin and adrenocorticotrophin, and also the interstitial-cell-stimulating hormone and luteotrophin. On the other hand, the follicle-stimulating hormone, which stimulates the growth and maturation of gonads, is mainly morphogenetic and so is the luteinizing hormone, since it promotes growth of the corpus luteum but does not stimulate it to secrete. The use of 'gonadotrophic' for all these four does not distinguish between their actions. The best known example of an endocrinokinetic hormone from an invertebrate is that which comes from the insect brain and stimulates the secretion of ecdysone.

D. B. Carlisle

Marine Biological Association Laboratory,
Plymouth.

Penelope M. Jenkin

Department of Zoology,
University of Bristol.

Nov. 25.

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Changes in the Osteoblastic and Mitochondrial Population of Aging Periosteum

Early investigators[1–4] interested in the physiology of bone have described filamentous, rod- and granular-shaped mitochondria in osteoblasts from a variety of animals both *in vivo* and *in vitro*. An apparent relation between the number of mitochondria and osteoblastic activity was also pointed out. Pritchard[5] arrived at the same conclusion. Within the past decade numerous biochemists have revealed a good many of the substrates and enzymes involved with intermediary metabolism associated with mitochondria. More recently, cytochrome oxidase and succinic dehydrogenase activity of the periosteum was studied in normal and traumatized rat femora from birth to old age[6,7]. The results showed high levels of activity of respiration becoming more elevated at the period of maximum rate of bone formation. Following this period of intense activity (5 weeks of age), a sharp drop occurred to a low level, which was maintained throughout the life of the animal. Trauma to the femora stimulated a response of respiratory activity, however, the response becoming progressively less with increasing age. Changes in respiratory enzyme-levels are fundamental to the activity of cells in general, because these enzymes are part of the aerobic hydrogen transport system from which rich-energy-bond phosphate is generated.

Since cytochrome oxidase and succinic dehydrogenase have been shown to be associated with mitochrondria[8], it was concluded that perhaps the changes in enzyme, noted with increasing age, reflected mitochondrial variations which resulted in changes in osteoblastic population. These alterations will be expected to effect the physiological functions of the periosteum in the processes of growth of bone and healing of fractures.

The experiments were performed to study the following: (1) quantitative changes in the osteoblastic population and their mitochondrial complement during the process of aging; (2) to relate these results to the previous respiratory enzyme findings; (3) to furnish a base-line for future studies of cytological changes associated with healing of fractures from birth to old age.

Long–Evans strain rats were used in the experiments and were divided into six age-groups containing ten animals each. Group I consisted of 1 week-old rats, Group II, 5 weeks-old, Group III, 8 weeks-old; Group IV, 26 weeks-old; and Groups V and VI, 52 weeks- and 104 weeks-old animals, respectively. Each group was subdivided into males and females. Intact femoral periosteum was obtained by shaving the mid-shaft of each bone. This method ensured the histological and cytological integrity necessary for proper measurements and eliminated the need of decalcification. Frozen sections were made and stained with janus green *B*. Additional tissues were fixed immediately with Regaud's fixative. Paraffin sections were prepared and stained with Regaud's iron hæmatoxylin.

Periosteal osteoblasts and their mitochondrial complement in contact with the preosseous zone were counted at each age. Special precautions were taken to count only the mitochondria of intact, non-distorted, free osteoblasts. The numerical data were evaluated for significance by statistical methods using Fisher's t, having a critical ratio of 2·797 and a probability of $P < 0{\cdot}01$.

Counts made of the number of periosteal osteoblasts adjacent to the preosseous zone revealed a slight

Table 1. A Comparison between the Average Number of Osteoblasts per Unit Area and the Average Number of Mitochondria per Osteoblast at the Femoral Periosteum of Rats of Various Ages

Age (weeks)	1	5	8	26	52	104
Average number of osteoblasts per unit area ± S.E.	10·2 ± 0·5	10·6 ± 0·4	10·5 ± 0·2	2·8 ± 1·9	3·5 ± 0·5	1·1 ± 0·2
Average number of mitochondria per osteoblast ± S.E.	28·4 ± 1·3	37·1 ± 2·4	16·8 ± 0·9	17·9 ± 1·4	9·5 ± 0·6	3·3 ± 0·5

S.E., standard error of the mean.

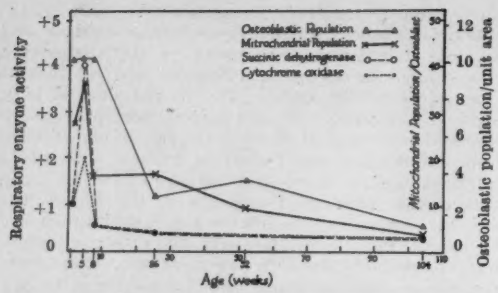

Fig. 1. A composite graph showing the relation between the shape of several curves at various ages. Note that the respiratory enzyme curves (succinic dehydrogenase and cytochrome oxidase) are similar to the mitochondrial population curve, and that the fall in respiratory enzyme activity and mitochondrial numbers precedes the disappearance of the osteoblasts from the periosteum

increase from birth to 5 weeks of age. This number was maintained to about 8 weeks of age, after which time there was a very significant fall in the number of osteoblasts in this region to 26 weeks of age. The following groups revealed a continued but less-severe diminution in the number of osteoblasts (Table 1). The mitochondrial complement of these cells also varied coincidentally with the variations noted in the number of osteoblasts. Mitochondria increased in number from birth to 5 weeks of age; however, only half this number was found by 8 weeks of age. The following ages revealed a progressive reduction in the mitochondrial complement (Table 1). A comparison of the number of osteoblasts, mitochondria and previously reported respiratory enzyme activity of the osteoblasts is given in Fig. 1. Morphological variations in osteoblasts were seen to occur following the numerical changes described (Fig. 2).

The results showed that a quantitative relation between the number of mitochondria and osteoblastic activity does exist, as has been pointed out by earlier investigators. It has also been shown that these changes precede the loss in the number of osteoblasts at the preosseous zone. Additional support is given by a comparison with respiratory enzyme findings[6,7], which were seen to parallel the mitochondrial changes. It is interesting to note that the reported drop in respiratory enzymes occurred also before the fall in the number of osteoblasts and that the greatest enzyme activities, number of mitochondria, and osteoblasts occur coincidentally with the period of maximum rate of bone formation in the rat. It appears, therefore, that during the processes of periosteal aging, biochemical changes are occurring associated with the mitochondrial complement of osteoblasts which are intimately involved with the long-recognized variations in the rate of bone growth and the observed morphological changes seen in ageing periosteal osteoblasts.

E. A. Tonna
N. Pillsbury

Department of Histo-Cytochemistry,
Hospital for Special Surgery,
New York.
Nov. 13.

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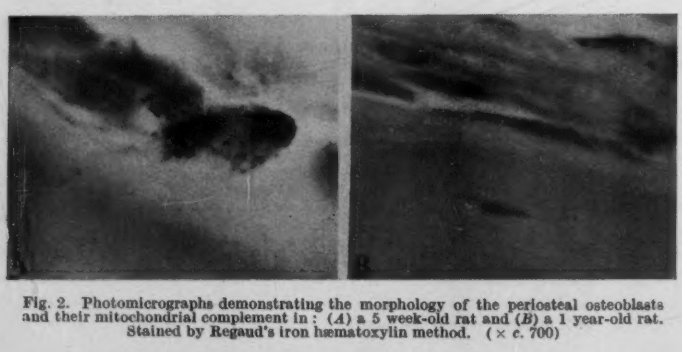

Fig. 2. Photomicrographs demonstrating the morphology of the periosteal osteoblasts and their mitochondrial complement in: (*A*) a 5 week-old rat and (*B*) a 1 year-old rat. Stained by Regaud's iron hæmatoxylin method. (× *c.* 700)

BIOLOGY

Differential Response of Male and Female Adults of *Trogoderma granarium* Everts towards Sterilizing Doses of Gamma-radiation

During a recent investigation into the tolerance of adults of *Trogoderma granarium* Everts to sterilizing doses of γ-radiation, it was decided to separate the sexes before treatment.

Previous work by Cornwell *et al.*[1] had shown that most insect pests of stored products could be sterilized by a dose of 6×10^3 rep. (In the present work, the rad has been used as the unit for measuring radiation. This unit has been adopted internationally for use in biological work in preference to the roentgen (r.), and the rep, both of which have been used in other works cited. The numerical differences between the three units are sufficiently small to enable reasonably accurate comparisons to be made between the results of others and the present work.

The work described here is a result of several trials with the adults of *T. granarium* which have shown that a dose of 5×10^3 rads (administered from a cobalt-60 source at 570 r. per min.) is sufficient to sterilize only the females of the species. The males (Table 1) are able to retain some fertility at dosages up to 15×10^3 rads, which is three times that required to sterilize the females.

It is interesting to compare this result with those of Bushland and Hopkins[2], who investigated the resistance of screw-worm flies, *Callitroga hominivorax* (Coq.), towards sterilizing doses of γ-radiation. In that case (where the insects were irradiated as pupæ) the sexes were also treated separately. In direct contrast to the present work, however, it was discovered that the males required less γ-radiation than the females to render them sterile. The males, in fact, were almost completely sterilized by a dose of $2{\cdot}5 \times 10^3$ r. given on the seventh (penultimate) day of the pupal stage. On the other hand, females laid eggs (which yielded a 50 per cent hatch) after being given a dose of 4×10^3 r. on the seventh day after pupation.

Table 1. Number of Larvæ present at each Observation. After Treatment, except at the Highest Dosage, Seven Males were confined with Twice their Number of Normal Newly Emerged Females

Days after exposure of males	No. of larvæ present following γ-irradiation of males (dose in rads)			
	0	5,000	10,000	15,000*
3				
4				
5	5			
6	10	2	1	
7	30	4	3	
8		6	7	
9				
10		20		3
11		20	14	5
12			14	8
13		26	14	
14			16	
15				
16			16	
17			20	
18				
19			20	
20				
21	40	26	20	8

* Thirty males were irradiated and confined with thirty females.

Table 2. Number of Males found dead at each Observation

Days after exposure	No. of males dead after dose (rads)			
	0	5,000	10,000	15,000
4	4	1		
5	2	1		4
6	0	1	1	5
7	1	3	4	5
8		1	2	12
9				3
10		26		1

The adults of *T. granarium* were irradiated immediately on emergence from the pupæ. Thus the difference between the resistance in the two sexes, when compared with *C. hominivorax*, cannot be attributed to irradiation of the insects at widely different stages in the development of the two species.

Some of the results (Table 2) indicate a possible increase in life-span following irradiation, and it is hoped to investigate the significance of such results in future work.

G. C. Carney

Department of Agricultural Zoology,
School of Agriculture,
University of Durham,
King's College,
Newcastle on Tyne.
Oct. 19.

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Measuring Productivity in Breeding of Small Animals

It is nearly always desirable, when breeding animals like mice for laboratory use, to select for high productivity, that is, for the largest number of healthy offspring per dam in a given time. This is a function of litter size and 'pre-natal interval'; the time between exposure to the chance of mating and the birth of the first litter, or the time between the births of successive litters. The pre-natal interval is only of interest for monogamous pairs which are never separated.

Carter[1] suggested that the measure of productivity be the number of mice born and weaned, divided by the number of prenatal days. This index will be less than 1·0, occasionally less than 0·1, and to be useful it will contain two significant figures. Later it was suggested[2] that the reciprocal of Carter's index be used which will be a figure greater than 1·0 and occasionally greater than 10·0—also needing to have two significant figures. Carter's index is inconvenient owing to the decimal point; but it varies indirectly with the productivity. The other figure is more convenient but still contains a decimal point; however, it varies inversely with the productivity, which is an added inconvenience.

Thanks to a suggestion made by D. S. Falconer (personal communication), we now propose to use Carter's index multiplied by 100 as a measure of productivity, and to call this index *Q*. It will always be less than 100, and in most cases more than 10: a typical range will be between 15 and 35. No decimal points are involved. We further propose to call *Q* the 'index of productivity', and to calculate it cumulatively from the date of the first exposure to the chance of mating to the birth of successive litters up to the last. This index gives, in a convenient form, a current measure of productivity of pairs of animals, on which selection can be based.

W. Lane-Petter
Annie M. Brown
Margaret J. Cook
G. Porter
A. A. Tuffery

The Laboratory Animals Centre,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton, Surrey.
Dec. 2.

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An Unusual Strain of *Escherichia coli* in Septicæmia as an example of the Transformative Effect of Symbiosis

A 54-year-old male patient was admitted to hospital with signs of septicæmia and obstructive jaundice after having suffered from gall-bladder troubles for the past five years. By blood culture in brain–heart infusion broth a rich growth of a highly polymorphous Gram-negative organism was obtained (Fig. 1, top) which gave the typical biochemical reactions of an *Escherichia coli* and a positive hæmagglutination test. The macroscopic appearance of the colonies also did not differ from a slightly mucoid *E. coli* culture. No dwarf colonies were found. The polymorphism (filaments with broad swellings, globular and club-shaped forms) was most marked on a culture 24–48 hr. old on 5 per cent rabbit blood agar. After three days the majority of the filaments had broken up into chains of rods and the bizarre forms had disappeared. This aberrant morphology persisted for ten months if the strain was subcultured only on blood agar. After this time it grew in more or less uniform short filaments. If subcultured exclusively on plain agar, it reverted after five months to the typical size and shape of *E. coli*. No morphological change could now be brought about by transferring it from plain to blood agar.

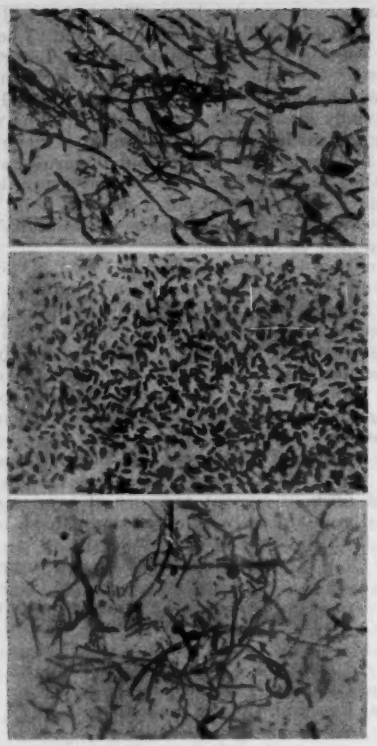

Fig. 1. Top, *E. coli* from blood, shortly after isolation, showing filamentous and swollen forms in a 24-hr. culture on blood agar; middle, bacillary form of the same strain, as in top, after five months subculturing on plain agar; bottom, the same bacillary variant as in middle, re-transformed into the aberrant form under the influence of a *Paracolon* strain. (× *c.* 670)

The patient's serum agglutinated the strain up to a dilution of 1/5,000, but the usual routine agglutinations (*Salmonella*, *Proteus X*, *Brucella* and *Leptospira*) were negative. Fifty control sera gave either negative results or titres below 1/100.

The strain was forwarded to the Statens Serum Institute, Copenhagen, for further investigation. The *coli H*-antigen 6 was found while the *O*-antigen could not be determined.

As a cholecystography showed an obstruction of the gall bladder, the patient was operated on and two stones removed from the common bile duct. Cultures on McConkey plates from bile obtained from the inflamed gall bladder during operation revealed the presence of scanty colonies of the same polymorphous *E. coli* as found in the blood together with many colonies of a quite different *Enterobacterium*. The latter one grew in short rods of uniform size and shape, formed on iron-triple sugar agar acid and gas only at the butt, utilized citrate, split urea and produced ammonia and indole but no hydrogen sulphate. It readily formed acid and gas from glucose and mannitol, but slowly from lactose and salicin. Saccharose, adonitol and inositol were not fermented. The hæmagglutination test was negative. On solid media, the growth was luxuriant but otherwise was not characteristic.

This second strain does not fit biochemically into any of the recognized types of Enterobacteriaceae. Belonging to the slow lactose fermenters, it might still be best to call it a *Paracolon bacterium*, although this group has lately been abolished by some authors[1].

The fact that the two strains were found together in the bile suggested the idea that this symbiosis was responsible for the morphological change of the *E. coli*. Therefore, we tried to re-induce the aberrant growth in its daughter strain from plain agar, which had changed back to the typical bacillary form, by culturing it together with the *Paracolon* in a liquid bile medium and by subsequent isolation on McConkey plates. This method failed because the *Paracolon* overgrew and destroyed the *E. coli* within two or three days. In the gall bladder, the *E. coli* was probably protected by mucus and exudate against antagonistic environmental influences, and in the intestines the *Paracolon* might not have been numerous enough to eliminate completely the *coli* strain. Our experiments succeeded, however, as soon as we applied a modified cross-streak technique, generally used for investigating antibiotic effects.

A segment of a blood agar plate was thickly inoculated with the *Paracolon* strain and incubated for two or three days. Now, a suspension of the bacillary *coli* strain was prepared and streaks were drawn with it perpendicularly to the edge of the *Paracolon* growth. Similar streaks were made on a separate blood agar plate as controls. After 24-hr. incubation, the controls showed rich uniform growth without morphological change (Fig. 1, middle). On the plate previously inoculated with the *Paracolon*, the growth of the *E. coli* was considerably inhibited but not completely abolished up to a distance of 20 mm. from the edge of the *Paracolon* growth and became gradually richer farther away. Morphologically, the bacillary variant was re-transformed to its previous filamentous form with its bizarre shapes (Fig. 1, bottom). This transformation occurred in the partly inhibited zone as well as in the uninhibited one.

Yet subcultures from the streaks on a fresh blood agar reverted to the bacillary form after 24 hr. if they were made within the first few days after the transformation into the aberrant form had taken place. If, however, the plates were sealed with 'Plasticine' in order to prevent exsiccation and the *E. coli* was kept in association with the *Paracolon* for three to four weeks, the filamentous phase proved to be much more stable in repeated subcultures on blood agar and it took about one month before it reverted to the bacillary form.

Neither inhibition nor morphological alterations could be induced by this method in a number of typical *coli* strains from stools.

It appeared to be most likely that the morphological change was brought about by a weakly antibiotic substance, probably a colicine[2,3], produced by the *Paracolon* and diffusing through the agar. Filament formation has been observed under the influence of growth inhibitors[4] but the organisms revert rather quickly to their original size as soon as they are brought back to normal conditions. We have seen the same in our transformation experiments of short duration. However, if subcultured on blood agar before a reversion to the bacillary phase occurred, our strain represented a fairly stabilized morphological variation, manifesting itself regularly at a certain period of growth by taking shapes which were similar to the *L* forms of *E. coli*[5]. Yet it did not grow in micro-colonies as is characteristic for true *L* forms. The comparative stability

of our variant was obviously brought about by a long-lasting symbiosis with the *Paracolon*. This could be reproduced, to a certain extent, by the association of the two strains for some weeks.

So far as we are aware, this is the first time that the induction of a rather stable transformation of a bacterial strain by its symbiosis *in vivo* with another one has been recognized as such and could be experimentally reproduced. A detailed account of our experiments is to be published elsewhere.

We wish to express our thanks to Dr. F. Ørskov, Copenhagen, for examining the *coli* strain serologically and to Dr. H. Salomon, Haifa, for preparing the photomicrograms.

R. Reitler
M. Teicher
A. Valero

Government Hospital 'Rambam',
Haifa, Israel.
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An Antifungal Substance in the Corn Plant and its Effect on Growth of Two Stalk-rotting Fungi

In a recent paper on root and stalk rot of field corn in south-western Ontario[1], we reported that stalk-rotting fungi do not invade the stalks of young corn plants. Subsequent experiments have been designed to investigate whether or not young corn stalks contain substances which will inhibit the growth of these fungi. Loomis *et al.*[2] found resistance factors in corn which were effective against the European corn borer, *Pyrausta nubilalis* (Hubn.), as well as *Penicillium chrysogenum* and other organisms. Virtanen *et al.*[3] isolated antifungal factors from wheat, maize and rye which inhibited *Fusarium* sp., *Penicillium* sp. and a wide range of other micro-organisms. In the present communication results are presented which demonstrate that extracts from young corn stalks inhibit growth of two stalk-rotting organisms, *Fusarium moniliforme* Sheld. and *Gibberella zeae* (Schw.) Petch.

Stalks of 52-day-old corn (Pioneer 349) were cut into small pieces and frozen. To 2,000-gm. samples of the frozen material, 1,000 ml. of distilled water was added and the mixture was boiled for 15 min. Juice, extracted from the mixture under pressure, was concentrated to 1,000 ml. by boiling. An ether extract from this juice was assayed for antifungal activity by measuring its effect on growth of *Fusarium moniliforme* and *Gibberella zeae*, which were grown on dehydrated Czapek's agar plus extract for 72 hr. at 25° C. The diameters of the colonies of *Fusarium moniliforme* on eight plates of Czapek's agar (control) and on eight plates of Czapek's agar plus extract averaged 4·10 cm. and 2·23 cm., respectively. This represents an inhibition of growth of 45·6 per cent caused by the extract.

Growth of *Gibberella zeae* was inhibited by 22·5 per cent.

In ten other lines of corn investigated, inhibition of *Fusarium moniliforme* ranged from 56·5 to 63·9 per cent.

The results of these experiments strongly suggest that stalks of young corn are not attacked by *Fusarium moniliforme* or by *Gibberella zeae* because of the presence of an antifungal substance in the young stalks.

N. J. Whitney

Science Service Laboratory,
Canada Department of Agriculture,
Harrow, Ontario.

C. G. Mortimore

Experimental Farm,
Canada Department of Agriculture,
Harrow, Ontario.
Oct. 15.

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Balanus tulipiformis Ellis on the Atlantic Coast of France

The presence of *Balanus tulipiformis* Ellis on the Atlantic coast of Europe was recently recorded for the first time[1]. It was there noted that this species had been observed on the south-west coast of France from 1956.

It has since transpired that among the barnacles in the Musée de la Mer, Biarritz, there are two lots of *B. tulipiformis*, both found considerably earlier. These specimens had not been recognized as such, and had been labelled *B. amphitrite* Darwin. Samples from each of these exhibits have been examined by Dr. J. P. Harding, who has confirmed their identification as *B. tulipiformis*.

The earlier lot consists of about 30 young or half-grown specimens which had been thrown up on the beach at Messanges (Landes). They were collected by M. Paul Arné, some living and some dead, between 1923 and 1933. In the latter year, M. Arné left Messanges to take up the post of director of the newly opened Musée de la Mer, Biarritz, taking his collections with him. The later exhibit is a group of 10 or so large specimens, all joined together, which was found on a piece of rock brought up with a crayfish trap off Biarritz in 1950.

It is thus established that a quarter of a century has elapsed since *B. tulipiformis* was first found on the south-west coast of France.

B. S. Kisch

Saint-Jean-de-Luz,
Basses-Pyrénées,
France.
Dec. 9.

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Facultative Parasitism and Dietary Change in *Harmolita hyalipenne* Walker

Dietary change from carnivory to phytophagy has been previously reported from four Eurytomidae; by Nielson[1] from Denmark for an unidentified species of *Eurytoma* parasitic on the larvæ of *Cryptocampus angustus* Htg. in willow twigs; by Rimsky-Korsakow[2] for *Harmolita inquilinum* (the U.S.S.R.) parasitic on *H. rossicum* in the stems of rye; and by Phillips[3] for *Eurytoma pater* Girault and *E. parva* Girault[4,5]

Table 1

Square No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Controls		
No of females introduced	10	1	2	5	5	1	2	2	4	2	1	1	5	1	0	0	0
No. of males	4	1	0	0	4	1	2	2	3	3	1	1	5	1	0	0	0
No. of galls	73	0	0	0	53	0	0	4	36	18	0	2	0	0	0	0	0

parasitic on the wheat joint worm *H. tritici* Fitch in the United States. In each case the eurytomid larva parasitizes its stem-living host larva, and having destroyed this becomes phytophagous in order to obtain sufficient nutriment for the completion of its development.

During an investigation into the species of Eurytomidae infesting the couch grass *Agropyron repens* Beauv. in Britain, *Harmolita graminicola* Giraud was found to be the main species inducing the formation of apical stem galls, while *H.* (*Eurytoma*) *hyalipenne* exhibited a facultative parasitism and played only a minor part in gall formation.

The female *H. graminicola* oviposits in June or July in the zone of elongation immediately below the apical meristem of the couch grass, and the single larva emerging from the egg feeds on the plant tissue inducing the formation of a fusiform apical gall. Its larva is bright yellow in its later instars and is easily distinguished from the white late instar larva of *H. hyalipenne*.

H. hyalipenne reaches a peak of emergence a few days later than *H. graminicola*, the females laying their eggs immediately above the final node of the stem which, however, usually forms the base of a young gall of *H. graminicola*, and, under natural conditions, the infested stems are selected for oviposition. The eggs are dark brown in colour, and from each an extremely active larva emerges which immediately attacks and destroys the gallicolous larva of *H. graminicola*, this still being in an early instar. The amount of food obtained from this brief parasitic period is small and insufficient for future development so that the larva then turns to the plant tissue, which it consumes until the onset of diapause.

It was decided in 1958 to test experimentally whether this parasitic part of the life-cycle was essential, since collections made in the field showed occasional galls in which there was no trace of the remains of *H. graminicola* larva, while an active *H. hyalipenne* was feeding inside the gall.

A series of square-yard samples of couch grass were cleared of galls by careful inspection and cutting the grass of the growth of the previous years. Over each square a butter-muslin cover was placed mounted on a wooden frame, and a known number of male and female *H. hyalipenne* which had been reared in the laboratory were then introduced into each square in July and allowed to oviposit on the stems.

After two months the covers were removed and the grass from each square cut and examined in the laboratory, where the galls were counted, the figures being shown in Table 1.

From this it is apparent that if *H. hyalipenne* females are deprived of stems infested by the larvæ of *H. graminicola* they will still continue to oviposit and their larvæ develop fully although unable to undergo a parasitic phase in their life-cycle. The galls which these larvæ produce are identical with those of *H. graminicola*.

When considering the life-cycles of the Eurytomidae where dietary change was then known, Gahan[6] inferred that phytophagy was a recent specialization arising from a parasitic life-cycle. Although *H. hyalipenne* shows a similar dietary change, its ability to dispense with parasitism in its larval stages and to develop normally by phytophagy indicates that the reverse may be true and that larval parasitism in this group is a more recent specialization.

R. Bailey

Science Department,
Hatfield Technical College,
Hatfield, Herts.

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GENETICS

Selection for Relationships Opposite to those predicted by the Genetic Correlation between Two Traits in the House Mouse (*Mus musculus*)

The majority of the concepts of population genetics used in predicting possible progress in livestock breeding are derived from the known behaviour of single genes. An exception to this is the concept of the genetic correlation between two traits which is based on the statistical partitioning of their variances and covariance[1]. The genetic portion of these variances and covariance is then used to estimate a probable change in one trait when the second trait is selected for. Falconer[2] has indicated the validity of this process for a particular case in that he obtained similar values for the genetic correlation between length of tail and body-weight in mice of six weeks of age from an experiment in which the response of each was measured when the other was selected for.

The genetic correlation in Falconer's experiment could be the result of pleiotropy or of linkage. If it resulted from pleiotropy of only a proportion of the genes concerned, then it should be possible to select for genes which affect tail length or body-weight only. A suitable selection method could then produce results opposite to those predicted by the genetic correlation. Some early results from such a selection experiment are available.

Initial stocks were obtained by inter-crossing the inbred lines *A*^{w} *C.57.Bl.* and *CBA* obtained from the Animal Genetics Section, Commonwealth Scientific and Industrial Research Organization, Sydney. All possible reciprocal crosses were made and the resulting progeny were then inter-crossed. Selection was started on mice from a second inter-crossing and made between all mice of the first litter in each generation with the only restriction being the avoidance of sib-matings. Males and females were selected separately and two litters were bred from each mating.

Selection was made on the deviations of the actual body-weight at six weeks from the expected value.

Table 1. THE MEAN LENGTH OF TAIL (CM.) AND BODY-WEIGHT (GM.) OF FIRST LITTER AT SIX WEEKS OF AGE FOR THE FINAL INTERCROSS AND FOR OFFSPRING OF SIX SELECTION GENERATIONS

Generation	Final intercross	S_1	S_2	S_3	S_4	S_5	S_6	Standard deviation S_6
Positive deviations				**Males**				
Tail length	8·6	7·8	8·5	8·2	8·0	7·7	7·8	0·46
Body-weight	20·9	16·6	24·3	23·2	22·3	23·3	25·4	1·61
Expected body-weight*	20·9	18·8	20·5	19·9	19·3	18·5	18·7	
Deviation	0·0	−2·2	+3·8	+3·3	+3·0	+4·8	+6·7	
				Females				
Tail length	8·3	7·7	8·1	8·2	7·8	7·5	7·9	0·23
Body-weight	17·3	16·7	19·6	20·0	18·4	20·0	20·7	1·58
Expected body-weight*	17·3	14·6	16·2	16·9	15·3	14·0	15·5	
Deviation	0·0	+2·1	+3·4	+3·1	+3·1	+6·0	+5·2	
Average litter size†	7·1	8·5	5·8	7·5	8·3	6·0	8·0	
Negative deviations				**Males**				
Tail length	8·6	8·1	9·0	8·9	9·0	9·6	9·7	0·42
Body-weight	20·9	19·1	23·2	20·4	23·0	22·7	21·1	1·50
Expected body-weight*	20·9	19·5	21·9	21·7	21·9	23·2	23·5	
Deviation	0·0	−0·4	+1·4	−1·3	+1·1	−0·5	2·4	
				Females				
Tail length	8·3	8·4	8·7	8·8	8·9	9·0	9·0	0·42
Body-weight	17·3	18·7	20·0	19·1	18·3	18·3	16·8	1·09
Expected body-weight*	17·3	17·7	19·1	19·4	20·0	20·1	20·3	
Deviation	0·0	+1·0	+0·9	−0·3	−1·7	1·8	−3·5	
Average litter size†	7·1	7·3	4·8	6·0	6·3	6·8	7·3	

*Estimated from genetic regression (see text), ♂♂ : $y = 2{\cdot}5X - 0{\cdot}7$; ♀♀ : $y = 4{\cdot}2X - 17{\cdot}5$

†Eighteen matings in the final intercross, four matings in each selection generation

This expected body-weight was estimated from the observed length of tail by use of the regression of body-weight on length of tail. Lines for positive and negative deviations were selected and for the first three generations the regression used was that calculated from the litters of previous generations. Subsequent selection has been by use of the regression within the first three generations of the positive and negative lines. Regressions for males and females were calculated separately.

Table 1 shows the length of tail and body-weights at six weeks for the first litters of six selection generations. The standard deviations shown are those for the offspring of the sixth generation. Selection for positive deviations has resulted in mice with shorter tails and greater body-weight than those arising from selection for negative deviations.

Estimates of the genetic correlations made from the initial crosses gave values of 0·66 for males and from 0·77 to 0·92 for females. For this type of estimate these values are in reasonable agreement with those of 0·57 and 0·62 obtained for both sexes by Falconer[2]. The genetic components of variance and covariance used to obtain the correlation were also used to calculate a regression of body-weight on length of tail. It is this regression which has been used to obtain the expected values of body-weight shown in Table 1.

These results show that it is possible to make fairly rapid progress in selection for relationships between two traits different from those predictable from the expected correlated response. An explanation based on a hypothesis that a proportion of the genes are pleiotropic for length of tail and body-weight is reasonable, but the alternative explanation of linkage of the genes concerned and selection of appropriate recombinants must be considered in this experiment. Mather and Harrison[3] have concluded that an observed correlated response in *Drosophila melanogaster* was the result of polygenic linkage. This is less likely in mice with their greater number of chromosomes and hence a lesser chance of any two genes being linked. However, if pleiotropy is concerned, then further selection for tail length alone should eventually give the appropriate correlated response of body-weight as the pleiotropic genes should still be present. If linkage was the cause of the original correlation and these linkages have been broken, as suggested by Mather and Harrison[3], then no correlated response of body-weight should follow continued selection for length of tail alone. These alternative possibilities are now being tested.

Whatever the underlying cause, it is clear that in this case the presence of a genetic correlation does not preclude the possibility of a rapid response to selection for various combinations of the correlated traits. Whether this is true in other cases may well depend on whether or not there is a unique physiological basis for the traits measured.

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F. COCKREM

Sheep Husbandry Department,
Massey Agricultural College,
Palmerston North.
Oct. 9.

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PSYCHOLOGY

Association between Psychomotor Behaviour and Delinquency

IN 1948, Davis[1] reported experimental evidence of significant association between manner of movement of controls in a simulator cockpit and (*a*) neurotic syndromes and (*b*) accident proneness among pilot trainees. In 1955, Venables[2] reported experimental results confirming Davis's finding (*a*) above, also (*c*) non-significant correlation of psychomotor response variable with general intelligence and (*d*) significant association of response pattern, on a task of graded difficulty, with subsequent job-dismissal in a small industrial group ($n = 18$).

The relation between psychomotor behaviour in this test situation (replicating the procedure described by Venables[2]), and subsequent delinquency (the criterion of delinquency, by Service classification, includes minor offences not thus classifiable by civil usage) in a large sample of Royal Air Force recruits to a relatively low-skilled trade, has been studied in a research for the Air Ministry, with results summarized below. (Theoretical implications are the subject of a separate report.) Initially, the finding of non-significant correlation between general intelligence and the psychomotor variate, and of the continuous unimodal distribution of the variate, was confirmed for the Royal Air Force population. Venables' preferred variate was the ratio of amplitude of manual responsive movement to time taken in making the movement; this measure has been used in logged form. The subject moves a knob in a slot, right or left and back to centre, in response to visual signals. The signals are presented in three series, of which the first (*A*) and last (*C*) are similar and relatively easy and the second (*B*) difficult for all subjects. The difficulty of response to *B*-series lies in the speed and complexity of presentation of the signals in the series as a whole. The stimuli for the responses which are scored, and the type of responsive movement required, are the same in all three series. The subject does not see the record of his movements.

Response to the three series tends to be made at a characteristic score-level, so that the series scores are correlated positively with a high degree of significance (product-moment r in a 20 per cent random sample of all subjects tested to a given date with $n = 251$ were: AB, $+0{\cdot}88$; AC, $+0{\cdot}87$; BC, $+0{\cdot}87$). Some individuals tend to reduce and some to increase score with change of difficulty of task; the direction of change has been found to be significantly associated with temperamental factors in the previous researches quoted. In this investigation both the general score-level and the degree and direction of the difficulty gradient were found significantly predictive of delinquency (service plus civil) over a period of up to 19 months subsequent to the administration of the test.

The results of two analyses are shown below, the first based on records of all tested entrants to training in the trade in question, over a given period ($n = 601$), the second a more detailed analysis of a smaller sample ($n = 157$). The crime record criterion was on a dual basis: (1) number of convictions, and (2) penalty awarded; categories ranged from zero on both counts to 3 or more convictions for offences of which at least one was indictable or penalized as serious by the Service authority. Psychomotor-test mean scores and variances were calculated for all groups and various combinations of groups. In the first study the non-delinquent group included: (1) men without record of conviction; (2) men convicted of one minor Service crime only; (3) men withdrawn from trade training for technical reasons only. The delinquent group included (1) men with several or serious offence records; (2) men withdrawn from trade training owing to unsuitability of character. Mean score differences were significant between the delinquent/non-delinquent groups but not within these groups. With the accumulation of additional records, the later study was restricted to group (2) of the non-delinquents and group (1) of the delinquents. More than 80 men were subjects of both studies, many appearing in different crime groups on the two occasions, owing to accumulation of individual crime records.

Table 1. Psychomotor Test Scores of Non-delinquent and Delinquent Groups: Initial Series and Difficulty Gradient Scores

Series	Non-delinquents $n = 567$ Mean	Non-delinquents $n = 567$ Variance	Delinquents $n = 34$ Mean	Delinquents $n = 34$ Variance	P of difference between means
A	195·6	1,494	220·1	1,694	$<0{\cdot}001$
$A-B$	3·24	420·4	13·03	629·2	$<0{\cdot}05$
$B-C$	−6·39	438·7	−14·03	346·8	$<0{\cdot}02$

Table 2. Psychomotor Test Scores of Non-delinquent and Delinquent Groups variously Classified: Differences between Mean Scores on Initial Series and Difficulty Gradients

Groups compared $n(1)-n(2)$	One minor crime with the rest 107/50		One crime with multiple crime 125/32		Minor crime with serious crime 119/38	
Series	Difference between $\bar{x}$	P	Difference between $\bar{x}$	P	Difference between $\bar{x}$	P
A	−19	$<0{\cdot}01$	−25	$<0{\cdot}01$	−22	$<0{\cdot}01$
$A-B$	− 5·6	$>0{\cdot}05$	− 7·4	$>0{\cdot}05$	− 9·5	$<0{\cdot}01$
$B-C$	+ 3·5	$>0{\cdot}05$	− 0·7	$>0{\cdot}05$	+ 6·2	$<0{\cdot}01$

It was found that the delinquent groups differed significantly from the non-delinquent both in manner of reaction to reduction of task difficulty ($B-C$ score), confirming Venables's finding[2], and also, more significantly, on all primary series scores, of which only A score is shown in Tables 1 and 2. They also differed significantly in manner of reaction to increase in task difficulty.

The psychomotor variate was interpreted by Davis as anticipatory tension, closely related to anxiety. It seems that anxiety is relatively chronic or diffuse in relation to the easier task, relatively specific or objective in relation to the more difficult task. By these investigations, association is established between psychomotor behaviour and delinquency, the association being interpreted in terms of level of chronic or diffuse anxiety and manner of reaction to change of specificity of the source of anxiety.

H. S. Anthony

Science 4,
Air Ministry,
London.
Nov. 11.

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Effect of Reduction of Stimulation on Consumption of Food, Intake of Water and Weight of Body in Rats

Recent work on factors determining the amount of general activity in animals indicates that such activity is related to: (*a*) the presence of stimuli previously associated with the diurnal cycle; (*b*) stimulus variety in the environment. However, the evidence is suggestive rather than conclusive, the main difficulty arising from the lack of specifiability and measurement of 'general' activity. We therefore investigated a readily measurable behaviour pattern, namely, eating and drinking.

Two hooded male rats, approximately 4 months old at the beginning of the experiment, were tested over a period of six months. The apparatus consisted of a cage measuring 13½ in. long, 9½ in. high and

Table 1

Trial	Unrestricted environment				Trial	Restricted environment			
	No. of responses	Food consumed (gm.)	Water intake (c.c.)	Weight change (gm.)		No. of responses	Food consumed (gm.)	Water intake (c.c.)	Weight change (gm.)
Rat 1					Rat 1				
1	733	18·4	9·5	10	2	555	13·8	7·8	0
3	702	17·5	8·8	5	4	527	13·2	7·5	10
5	712	17·8	8·8	15	6	526	13·1	7·8	0
7	721	18·0	9·0	5	8	529	13·2	7·8	0
9	718	17·9	9·0	0	10	530	13·2	7·5	0
Mean	717·0	17·9	9·0			533·4	13·3	7·7	
Rat 2					Rat 2				
1	620	15·5	8·0	35	2	427	10·7	6·5	5
3	632	15·8	7·2	0	4	440	11·0	7·0	0
5	605	15·1	7·5	10	6	468	11·7	6·8	5
7	597	14·9	8·0	10	8	452	11·3	6·8	0
9	609	15·2	7·5	0	10	443	11·1	6·5	0
Mean	612·6	15·3	7·6			446·0	11·1	6·7	

8 in. wide, fitted with a lever. When the lever was pressed, powdered food (0·025 gm.) was delivered from a concealed food hopper into a recessed food trough placed to the left of the lever. The apparatus could be enclosed in a sound-proofed box which was suspended in a sound-proofed chest. The apparatus (including control equipment) stood in the experimenter's office. The inner box was ventilated by an air pump and was constantly illuminated by a 15-watt lamp. The apparatus and subjects were kept for 4 days inside the chest, and then for 4 days the apparatus was placed, exposed, on top of the chest. Thus the animal was alternately subjected to reduction (environmental restriction) and variety of stimulus.

Table 1 gives the results for animals 1 and 2 respectively. Each trial period lasted for 4 days. The results cover the last 40 days of the experiment and consist of the mean daily frequency of pressing of the lever, the mean daily amount of food consumed (gm.), the mean daily amount of water drunk, and the change in weight during the trial, under each of the experimental conditions.

Statistical tests support the conclusion that the effect of reduction of stimulation (restricted environment) is to reduce the animal's food consumption and intake of water, as well as to inhibit temporarily gain in weight. During the restricted environment phase of the experiment, the food consumption of animal 1 fell off by 25·7 per cent and intake of water by 15 per cent. The food consumption of animal 2 fell off by 27·2 per cent and intake of water by 12·4 per cent.

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KARL WEISS
HARRY M. B. HURWITZ
Department of Psychology,
Birkbeck College,
London, W.C.1. Nov. 7.

Threshold Values for Taste in Monozygotic Twin Calves

ELECTROPHYSIOLOGICAL studies have shown that specific taste receptors are distributed in definite topographical arrangements on the tongue of man and laboratory animals[1,2]. More recently, similar studies have been extended to include domesticated ruminants[3]. Taste discrimination has been demonstrated in a variety of laboratory animals[4,5] and using this type of preference test, threshold values for rejection and acceptance of a variety of sapid substances have been established for a number of different species.

Discrimination tests for taste have now been carried out using monozygotic twin calves as subjects. Monovular calves were chosen for investigation for two reasons; first, in order to extend knowledge of sensory discrimination and especially of the sense of taste to another species, and secondly, to find out whether monovular cattle twins show the same close similarity in the inheritance of physiological sensory thresholds as they do for anatomical and biochemical factors[6].

The 'two-choice' discrimination tests for cattle were carried out in a manner like that devised for the goat[7]. Each calf was kept in a similar specially fitted stall, and feeding and management were standardized so far as possible. The front of each animal's stall allowed access to two identical polythene buckets suitably fixed at ground-level; normally, these buckets were kept filled with tap water. For the test a known concentration of test substance in solution in tap water was placed in one bucket and tap water only in the other bucket. The position of the test solution was reversed the next day to offset any bias due to visual or behavioural cues. For each single concentration of solution the aggregate amount consumed in the right and left position was used in assessing the results. The amount of test solution taken during the two test periods of nine hours on consecutive days was calculated as a percentage of the total fluid intake, that is, volume of test solution ingested and volume of water ingested.

The results for the whole group of calves and different sapid solutions are presented graphically in Figs. 1–4. It can be seen that high concentrations of test substance are usually rejected almost completely, the small amounts taken being only sufficient to stimulate the animals' taste receptors. The rejection threshold has been placed arbitrarily at the lowest concentration where not more than 20 per cent of the total fluid intake has been consumed. The ideal acceptance threshold, the level at which animals do not discriminate between test solution and tap water, would be the level when 50 per cent of the total fluid intake is the test solution. In practice this value probably lies between 40 and 60 per cent of the total fluid intake. The mean values for rejection and acceptance thresholds for all calves for the four normal taste modalities of sweet, sour, salt and

Table 1. Gustatory Thresholds of Calves to the Four Taste Modalities of Bitter, Sour, Salt and Sweet. The Number in Brackets refers to the Number of Animals Tested

Substance		Threshold concentration (moles)	
		Rejection	Acceptance
Quinine dihydrochloride	(12)	0·000097	0·000024
Acetic acid	(8)	0·026	0·0083
Sodium chloride	(8)	0·42	0·105
Glucose	(8)	—	1·11

bitter are shown in Table 1. In the case of sweet (glucose) taste sense there does not appear to be a true rejection threshold value.

The threshold values obtained between the different animals of the group shows a very much wider range than that shown between the individuals of a twin-set. The mean of the results for all the calves tested is plotted in Figs. 1–4 together with the values for the individuals of a monozygous twin-set. In an attempt to make the tests more rigorous and to eliminate environmental factors the individual members of the set of twin calves shown in Figs. 1–4 were separated by a distance of 15 miles, one of each set being housed at Potters Bar and the other at Camden Town. (The broken line in Figs. 1–4 is the mean of all the calves tested. The open and closed circles are the results obtained from the individuals of a monovular twin set, one of which was housed at Potters Bar and the other at Camden Town.) Furthermore, the test solutions were offered on different days at Potters Bar and Camden Town and the personnel dealing with the animals was different at the two places. The 'within-set' taste discrimination and consequently the close similarity of gustatory thresholds of calves

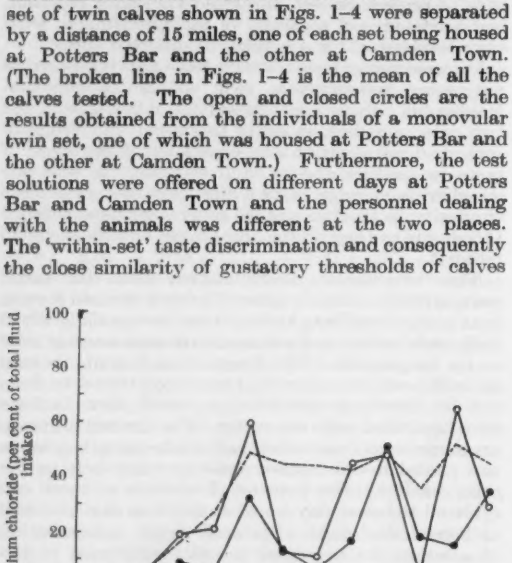

Fig. 1. Sodium chloride taste preference tests

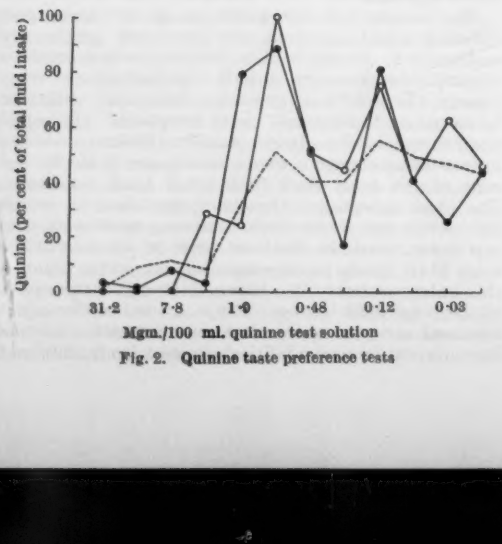

Fig. 2. Quinine taste preference tests

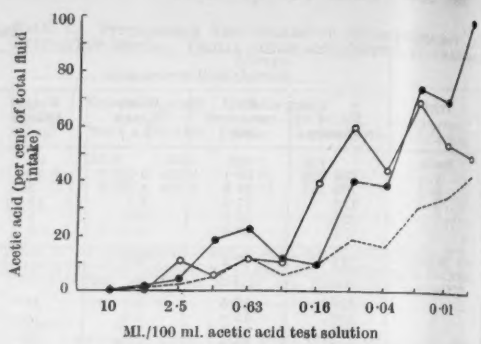

Fig. 3. Acetic acid taste preference tests

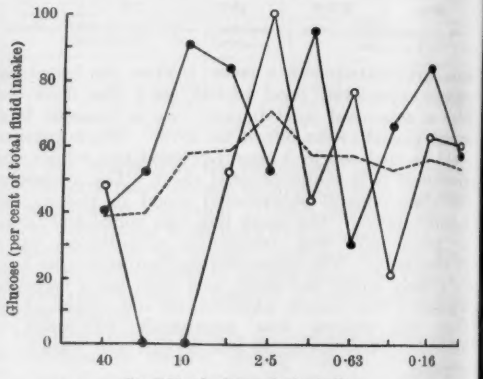

Fig. 4. Glucose taste preference tests

is shown in Figs. 1–4. It would appear from the close relationship of the 'within-set' differences obtained that taste discrimination was almost entirely genetical in origin, and that environmental factors play only a minor part in this type of behavioural reaction.

These results afford further evidence of the value of monozygotic twins as subjects for experimental research in the larger animals. Monovular twin cattle will obviously be the experimental animals of choice in the design of experiments to study physiological sensory mechanisms and nervous pathways in ruminants, for each pair will provide a control and experimental subject, thus reducing the high costs associated with experimental investigations of these large ungulates.

This work will be reported in detail elsewhere. We wish to express our thanks to the Agricultural Research Council for financial assistance and to B.O.C.M., Ltd., for generous supplies of animal foods.

F. R. Bell
H. L. Williams

Departments of Physiology and Animal Husbandry,
Royal Veterinary College,
London, N.W.1.
Nov. 25.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 2

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. I. C. Cocking: Presidential Address.

UNIVERSITY OF LONDON INSTITUTE OF EDUCATION (in the Assembly Hall, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. H. J. Eysenck: "The Principles of Conditioning and the Theory of Education".*

SOCIETY OF CHEMICAL INDUSTRY (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. D. A. Wright: "The Selection and Control of Semiconductors for Different Applications".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Dr. Waldo Lyon: "Trans-Polar Voyage of U.S.S. *Nautilus*".

Tuesday, February 3

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. L. S. Palmer: "Examples of Geoelectric Surveys".

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal discussion of the paper by Mr. N. F. Rieger: "An Experimental Investigation of the Pressurized Air-Lubricated Journal Bearing in the High-Speed Range".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (joint meeting with the BIOMETRIC SOCIETY, in the Small Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Scientific Papers.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. F. R. G. Gimblett: "Polymers and Derivatives of Phosphonitrile Chloride".

Wednesday, February 4

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. D. G. R. Bonnell: "The Mechanization of Building Constructional Processes" (Alfred Bossom Lecture).

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. D. Anson: "A Radioactive Corrosion Probe".*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. D. H. Tullis, Mr. L. C. Neill and Mr. A. T. Henderson: "Control of Marine Organisms in a Salt-Water Cooling System".

UNIVERSITY OF LONDON (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. De Santillana (Massachusetts Institute of Technology): "Space in Antiquity and the Renaissance".* (Further lecture on February 6.)

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. G. D. Rosen: "Practical Implications of the Laboratory Evaluation of Protein Quality".

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Inaugural Meeting.

SOCIETY FOR ANALYTICAL CHEMISTRY (in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "The Estimation of Pesticide Residues".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the SOUTH-WEST ESSEX COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, at the South-West Essex College of Technology, Forest Road, London, E.17), at 7.30 p.m.—Dr. S. F. Birch: "Petroleum Chemicals".

Thursday, February 5

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. A. J. Lloyd: "Water for the Swiss Jura".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. A. H. Cooke, Mr. J. H. E. Griffiths, Mr. B. R. Judd, Mr. R. Lazenby, Mr. F. R. McKim, Mr. J. Owen, Mr. J. G. Park, Mr. M. F. Partridge and Mr. W. P. Wolk: "Exchange Interactions in Antiferromagnetic Salts of Iridium. 1: Paramagnetic Resonance Experiments. 2: Magnetic Susceptibility Measurements. 3: Theory of the Interaction".

INSTITUTE OF REFRIGERATION (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Dr. H. J. Goldsmid: "Thermoelectric Cooling".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. T. H. Mason, Dr. P. D. Aylett and Mr. F. H. Birch: "Turbo-Generator Performance under Exceptional Operating Conditions."

ROYAL ANTHROPOLOGICAL INSTITUTE (at 21 Bedford Square, London, W.C.1), at 5.30 p.m.—Dr. J. H. M. Beattie: "Neighbourliness in Bunyoro".

UNIVERSITY OF LONDON INSTITUTE OF EDUCATION (in the Assembly Hall, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. L. J. Lewis: "Partnership in Oversea Education"* (Inaugural Lecture).

ROYAL AERONAUTICAL SOCIETY (at Hatfield Technical College, Hatfield, Herts), at 6.15 p.m.—Mr. J. L. P. Brodie: First Halford Memorial Lecture.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the AGRICULTURE GROUP, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. Bond and Mr. R. Cooper: Two papers on "Nitrogen Fixation".

POLAROGRAPHIC SOCIETY (at "The Duke of York", 8 Dering Street, London, W.1), at 7 p.m.—Informal Discussion Meeting on "Some Polarographic Problems Encountered in the Semi-Conductor Field" introduced by Dr. V. J. Jennings.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the KINGSTON TECHNICAL COLLEGE CHEMICAL SOCIETY, at the Kingston Technical College, Fassett Road, Kingston), at 7 p.m.—Prof. M. Stacey, F.R.S.: "Fluorinated Hydrocarbons".

Friday, February 6

UNIVERSITY OF LONDON (in the Meyerstein Lecture Theatre, Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Prof. L. T. Samuels (University of Utah): "Studies of Steroid Biosynthesis in Testicular Interstitial Cells".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Problems of Storing Transient Phenomena for Subsequent Analysis" opened by Dr. P. Bauwens and Mr. P. Styles.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. V. E. Cosslett: "Microscopy with X-Rays".

Saturday, February 7

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. R. A. Skelton: "Captain James Cook's Voyages in the South Seas and their Scientific Value".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (COMPUTATIONAL) (preferably with a degree and interested in the application of numerical methods to chemical problems) IN THE DEPARTMENT OF CHEMISTRY—The Registrar, The University, Manchester (February 7).

SENIOR LECTURER OR LECTURER (with special qualifications in pure mathematics) IN MATHEMATICS—The Registrar, The University, Manchester 13 (February 7).

PIG BREEDING ADVISOR (agricultural graduate with good post-graduate experience in the scientific aspects of animal breeding and a sound practical knowledge of pig breeding), for duties concerned with advising at farm level on pig breeding problems including those arising in connexion with pig recording and progeny testing—The Establishment Officer, Pig Industry Development Authority, Pida House, 15–17 Ridgmount Street, London, W.C.1 (February 10).

SENIOR RESEARCH ASSISTANT (preferably with a knowledge of electron optics and vacuum physics), for experimental research on the aberrations of electron lenses—The Secretary of the Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge (February 14).

LECTURER (with an honours degree in zoology, specialized in agricultural entomology, and experience in pest control and plant protection) IN ENTOMOLOGY in the School of Agriculture, Kumasi College of Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (February 18).

UNIVERSITY ASSISTANT LECTURER (preferably specialized in applied mathematics and theoretical physics) IN MATHEMATICS—The Secretary of the Appointments Committee of the Faculty of Mathematics, The Arts School, Bene't Street, Cambridge (February 18).

LECTURER, an ASSISTANT LECTURER, and RESEARCH FELLOWS IN THE DEPARTMENT OF PHYSICS—The Deputy Registrar, The University, Birmingham 15 (February 19).

ASSISTANT LECTURER IN GEOGRAPHY—The Registrar, The University, Leicester (February 21).

ASSISTANT LECTURER IN THE DEPARTMENT OF AGRICULTURE (CROP HUSBANDRY)—The Registrar, University College of Wales, Aberystwyth (February 21).

CHAIR OF GEOLOGY—The Registrar, The University, Leicester (February 21).

RESEARCH ASSISTANT (preferably with experience in electron microscopy, electronics and vacuum physics), to operate and maintain a Siemens electron microscope in the Metal Physics Group of the Cavendish Laboratory—The Secretary of the Department of Physics, Cavendish Laboratory, Cambridge (February 21).

RESEARCH FELLOWS (Ph.D. standard, with special aptitude for research in high or low energy nuclear physics) IN EXPERIMENTAL OR THEORETICAL NUCLEAR PHYSICS—The Registrar, The University, Liverpool (February 21).

CHAIR OF MATHEMATICS (THEORETICAL MECHANICS)—The Registrar, The University, Southampton (February 28).

LECTURER IN ZOOLOGY in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (February 28).

LECTURER OR ASSISTANT LECTURER IN BOTANY at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

READER IN CLASSICAL ARCHÆOLOGY—The Secretary of Faculties, University Registry, Oxford (February 28).

WILLIAM COCHRANE CHAIR OF METALLURGY tenable at King's College, Newcastle-upon-Tyne—The Registrar, University Office, 46 North Bailey, Durham (February 28).

LECTURER IN PHYSICS—The Secretary and Registrar, The University, Southampton (March 4).

LECTURER IN BOTANY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 6).

LECTURER (qualified in the field of palæontology, particularly micropalæontology) IN GEOLOGY at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, March 9).

POSTDOCTORAL FELLOW (with a Ph.D. degree in physical chemistry, preferably high polymer chemistry, radiation chemistry or some branch of reaction kinetics) IN THE DEPARTMENT OF PHYSICAL CHEMISTRY—Prof. F. S. Dainton, School of Chemistry, The University, Leeds (March 15).

RESEARCH FELLOWS (2) IN THE DEPARTMENT OF NUCLEAR PHYSICS, Research School of Physical Sciences, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 16).

SENIOR LECTURER (with an honours degree in engineering and preferably some knowledge or experience of industrial engineering) IN MECHANICAL OR INDUSTRIAL ENGINEERING at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 16).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOW IN CHEMISTRY, OR PHYSICS—The Secretary, The University, Exeter (March 23).

LECTURER IN BOTANY (PLANT PHYSIOLOGY) at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, March 31).

SENIOR LECTURER IN SOIL MICROBIOLOGY, and a SENIOR LECTURER IN FARM MANAGEMENT at the Institute of Agriculture, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 31).

HEAD (male of high scientific attainment with wide research experience either in soil science or in a relevant basic subject such as physical chemistry) OF THE DEPARTMENT OF PEDOLOGY—The Secretary, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (April 6).

LECTURERS (2) IN BOTANY—The Secretary, The Queen's University, Belfast (April 10).

HEAD (with high qualifications and considerable research experience) OF THE DEPARTMENT OF PHYSIOLOGY—The Director, Institute of Animal Physiology, Agricultural Research Council, Babraham Hall, Babraham, Cambridge (April 30).

LECTURER (with a good honours degree in zoology and preferably some experience of teaching and research) IN ZOOLOGY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, May 30).

ASSISTANT LECTURER (graduate, preferably with teaching experience in physics) IN PHYSICS—The Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

CHEMIST or BIOCHEMIST (preferably with postgraduate experience) IN THE DEPARTMENT OF RADIOCHEMISTRY, for fundamental biochemical research involving the use of radioactive isotopes, and in particular C^{14} and tritium—The Secretary, National Institute of Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 59/2.

HEAD (graduate of a British university and experience in university teaching and research) OF THE DEPARTMENT OF CIVIL AND MECHANICAL ENGINEERING—The Clerk to the Governing Body, Battersea College of Technology, Battersea Park Road, London, S.W.11.

LECTURER IN MATHEMATICS, to teach up to B.Sc. General Degree standard, and an ASSISTANT LECTURER (with a degree or equivalent and preferably industrial and/or teaching experience) IN TELECOMMUNICATIONS, to teach up to the Final Certificate of the City and Guilds of London Institute and the R.T.E.B. Radio and Television Servicing Certificates—The Clerk to the Governors, Northern Polytechnic, Holloway, London, N.7.

RESEARCH ASSISTANTS (ACADEMIC) (with a good degree and preferably some research experience) IN THE PHYSICS DEPARTMENT, to take part in research in the field of vacuum physics and photoelectronics applied to nuclear physics, astronomy, etc.—Prof. J. D. McGee, Imperial College, London, S.W.7.

TECHNICIAN (with experience in bacteriological work) IN THE VIRUS RESEARCH LABORATORY, Lodge Moor Hospital—Prof. C. H. Stuart-Harris, The Royal Hospital, Sheffield 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

International Association for the Exchange of Students for Technical Experience (IAESTE), Great Britain. Report for the year ending September 1958 and a Survey of IAESTE in Great Britain, 1948–1957. Pp. 58. (London: International Association for the Exchange of Students for Technical Experience, 1958.) [1911

Fifteen Years of Semiconducting Materials and Transistors: a Bibliography. Compiled by N. L. Meyrick. Pp. iv+75. (Newmarket: Newmarket Transistor Co., Ltd., 1958.) [2011

Government of Northern Ireland: Department of Agriculture. Leaflet No. 32: Fowl Pox. Pp. 3. Leaflet No. 59: Bacilliary White Diarrhoea or Pullorum Disease. Pp. 3. (Belfast: Ministry of Agriculture, 1958.) [2111

Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 8: Biological Methods for the Evaluation of Rodenticides. By Dr. E. W. Bentley. Pp. iv+35+4 plates. (London: H.M. Stationery Office, 1958.) 3s. net. [2111

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 686, Vol. 242 (20 November 1958): The Natural History, Embryology, Larval Biology and Post-Larval Development of *Adalaria proxima* (Alder and Hancock) (Gastropoda Opisthobranchia). By Dr. T. E. Thompson. Pp. 1–58+plate 1. (London: Royal Society, 1958.) 20s. [2111

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series 1, Vol. 6, No. 2: A Field and Laboratory Investigation of Fish in a Sewage Effluent. By I. R. H. Allan, D. W. M. Herbert and J. S. Alabaster. Pp. ix+76+7 plates. (London: H.M. Stationery Office, 1958.) 21s. net. [2411

Snowdonia. (National Park Guide No. 2.) Pp. xii+104+17 plates. (London: H.M. Stationery Office, 1958.) 5s. net. [2511

Air Ministry: Meteorological Office. Geophysical Memoirs, No. 101: Upper Air Temperature Over the World. By N. Goldie, J. G. Moore and E. E. Austin. Pp. 228. (M.O. 631a.) (London: H.M. Stationery Office, 1958.) 30s. net. [2511

Factories Bill. Pp. ii+17. (Bill 7.) (London: H.M. Stationery Office, 1958.) 1s. 3d. net. [2611

Forestry Commission. Leaflet No. 19: Elm Disease *Ceratostomella ulmi*. Pp. 7. Revised. 9d. Leaflet No. 55: Voles and Field Mice. By Prof. F. W. Rogers Brambell. Pp. 11. 1s. net. (London: H.M. Stationery Office, 1958.) [2611

Flora of Tropical East Africa. Edited by C. E. Hubbard and E. Milne-Redhead. Melianthaceae. By Dr. B. Verdcourt. Pp. 7. 1s. 6d. Resedaceae. By J. Eiffers and P. Taylor. Pp. 6. 1s. 6d. (London: Crown Agents for Oversea Governments and Administrations, 1958.) [2611

Science and History. By Prof. Douglas McKie. (An Inaugural Lecture delivered at University College, London, 22 May, 1958.) Pp. 17. (London: H. K. Lewis and Co., Ltd., 1958.) 2s. 6d. net. [2611

An Introduction to Concrete: Notes for Students. Pp. 26. (London: Cement and Concrete Association, 1958.) [2611

Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1293: Quality of Surface Waters of the United States, 1953. Parts 9–14: Colorado River Basin to Pacific Slope Basins in Oregon and Lower Columbia River Basin. Prepared under the direction of S. K. Love. Pp. xi+372. 1.25 dollars. Water-Supply Paper 1350: Quality of Surface Waters of the United States, 1954. Parts 1–4: North Atlantic Slope Basins to St. Lawrence River Basin. Prepared under the direction of S. K. Love. Pp. xi+415. n.p. Water-Supply Paper 1370–B: Floods of October 1954 in the Chicago Area, Illinois to Indiana. By Warren S. Daniels and Malcolm D. Hale. Pp. vi+107–200. 35 cents. Water Supply Paper 1399: Surface Water Supply of Hawaii, 1954–55. Prepared under the direction of J. V. B. Wells. Pp. vi+140. 50 cents. Water Supply Paper 1430: Quality of Surface Waters for Irrigation, Western United States, 1954. Prepared under the direction of S. K. Love. Pp. viii+205+1 plate. n.p. Water-Supply Paper 1435: Surface Water Supply of the United States, 1956. Parts 3-A: Ohio River Basin Except Cumberland and Tennessee River Basins. Prepared under the direction of J. V. B. Wells. Pp. xii+561. n.p. Bulletin 1042–0: Geology and Coal Resources of the Walsenburg Area, Huerfano County, Colorado. By Ross B. Johnson. Pp. iii+557–583+plates 47–49. n.p. Bulletin 1062: Geology and Coal Resources of the Toledo-Castle Rock District, Cowlitz and Lewis Counties, Washington. By Albert E. Roberts. Pp. v+71+16 plates. 2 dollars. Bulletin 1086-B: Geophysical Abstracts, 173, April–June, 1958. By Dorothy B. Vitaliano, S. T. Vesselowsky and others. Pp. iii+85–206. 35 cents. (Washington, D.C.: Government Printing Office, 1958.) [1811

United States National Museum. Bulletin 193: Supplement 1—Publications of the United States National Museum, January 1947–June 1958. Compiled in the Editorial and Publications Division, Smithsonian Institution. Pp. 1+16. (Washington, D.C.: Smithsonian Institution, 1958.) [1911

Western Australia. Report of the Government Chemical Laboratories for the year 1956. Pp. 13. (Extract from the Report of the Department of Mines.) (Perth: Government Printer, 1958.) [1911

Memoria del V Congresso Internacional de Radiacion Cosmica, Guanajuato, 5 al 13 septiembre de 1955. Edición a cargo de Manuel Sandoval Vallarta. Pp. 565. (Mexico: Instituto Nacional de Investigacion Cientifica, 1958.) [2011

Die Entfaltung der Wissenschaft: Zum Gedenken an Joachim Jungius (1587–1657). (Vorträge gehalten auf der Tagung der Joachim Jungius-Gesellschaft der Wissenschaften, Anlass der 300. Wiederkehr des Todestages von Joachim Jungius.) Pp. 160. (Glückstadt: Kommissions-Verlag J. J. Augustin, 1958.) [2111

Institut des Parcs Nationaux du Congo Belge. Exploration du Parc National Albert, Mission d'Études Vulcanologiques. Fascicule 3: Le Volcan Mugogo. Par Marcel A. P. Verhaeghe. Pp. 29+4 plates. Exploration de Parc National Albert, Mission J. de Heinzelin de Braucourt (1950). Fascicule 5: Les Ossements Humains du Cité Mésolithique d'Ishango. Par François Twiesselmann. Pp. 125+14 plates. (Bruxelles: Institut des Parcs Nationaux do Congo Belge, 1958.) [2411

United States Department of Agriculture. Farmers' Bulletin No. 2125: Making and Preserving Apple Cider. Pp. 16. (Washington, D.C.: Government Printing Office, 1958.) 10 cents. [2511

Highway Safety and Traffic Control. Edited by John W. Gibbons. (Annals of the American Academy of Political and Social Science, Vol. 320, November 1958.) Pp. x+209. (Philadelphia: American Academy of Political and Social Science, 1958.) 2 dollars. [2511

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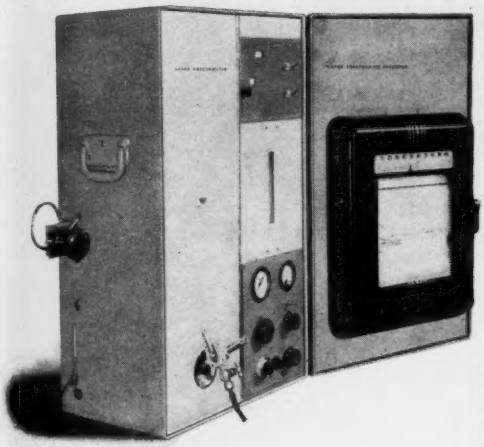

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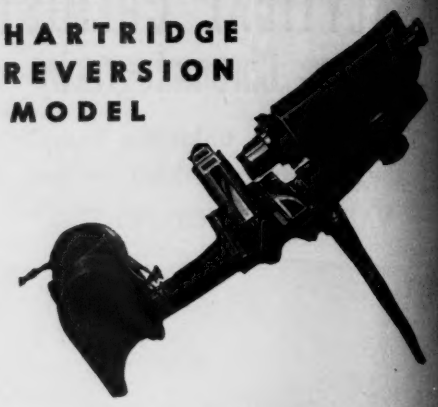

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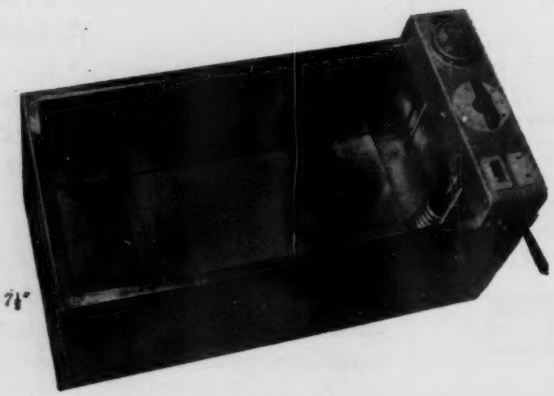

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